Production of edible flowers: irrigation and biotechnology[1]

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ABSTRACT

Garden pansy is a versatile gardening plant – it produces beautiful colorful edible-flowers with high value in gourmet cuisine. The use of irrigation and biotechnology in garden pansy cultivation can provide gains in flower productivity and nutritional value. The goal of this study was to evaluate the growth and edible flower production in garden pansy plants, submitted to different levels of irrigation and mycorrhizal inoculation. The experiment was conducted in randomized blocks in the 2 x 5 factorial design, with the presence and absence of mycorrhizal inoculation in combination with 5 levels of irrigation with 6 replicates, in a greenhouse. There was no significant interaction between the factors mycorrhizal inoculation and irrigation levels by the F test. Under the tested conditions, the mycorrhizal inoculation was unable to provide significant changes in the growth, development and flowering of garden pansy plants. It was concluded that no symbiotic efficiency was pointed out between the mycorrhizal fungus used and garden pansy plants. The best growth and yield results for cultivating and producing edible flowers of garden pansy were obtained at the 100% replenishment level of water evaporation.

Keywords: *Viola* × *wittrockiana*; floriculture; water deficit; mycorrhizal fungi.

INTRODUCTION

The market for edible-flowers is growing world-wide as it combines the beauty of the flowers with health, functional foods and new trends in the gourmet cuisine (Reis *et al.,* 2004; Kinupp & Lorenzi, 2014). Thus, its growing demand can mediate the economic development of flower producing countries, such as Brazil, through the identification of a new market niche (Rivas-García *et al.*, 2021).

The use of plants with edible flowers to compose gardens and landscapes are highly desirable, since the flowers bring not only beauty and balance to the environment, but they are a great source of antioxidant compounds and essential oils, being well appreciated in the high world gastronomy (Kinupp & Lorenzi, 2014; Santos & Reis, 2021). The edible flowers are highlighted, among functional foods, due to the presence of bioactive compounds, which are capable of neutralizing free radicals and contributing to a healthy and balanced diet (Gonçalves *et al.*, 2019b; Janarny *et al.*, 2021; Wu *et al.*, 2022).

Edible flowers of garden pansy (*Viola* × *wittrockiana* Gams.) are considered one of the favorites to be used in the elaboration and decoration of gourmet dishes, due to their varied color combination, velvety texture and slightly

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sweet taste (Kinupp & Lorenzi, 2014). The evaluation of agronomic procedures to improve the production and quality of the garden pansy is fundamental for development of its market.

Water management is one key factors for flower production. The growing pressure on the water resources implies, in terms of agriculture, in the need to seek more efficient irrigation management strategies. Water availability in the root zone impacts agricultural production qualitatively and quantitatively. About 97% of water uptake by the root system is lost by transpiration on leaves surface due to the continuous need for CO_2 absorption (Taiz & Zeiger, 2013). The water balance is a tool that can be applied to monitor the adequacy of the water supply in the soil throughout the crop cycle, as well as for the calculation of evapotranspiration. Several studies have been devoted to evaluating crop response to differentiated strategies for water supply management (Arévalo *et al.*, 2014; Álvarez & Sánchez-Blanco, 2015).

The use of biotechnology, especially arbuscular mycorrhizae (AMs), in plant production results in improvements in the physiological processes, nutrition, growth, development and protection of plants (Baum *et al.*, 2015; Chen *et al.,* 2018). Favorability in the water-plant relationship promoted by symbiosis with AMs can be considered the second major beneficial effect in plants after the nutritional effect. AMs promote changes in leaf elasticity, elevate leaf water potential and turgor, increase transpiration rate and stomatal conductance, reduce root resistance and promote root length and branching (Xu *et al.*, 2018; Pavithra & Yapa, 2018). However, a set of environmental factors, and factors related to the host plant may interfere in the formation, functioning and occurrence of AMs (Baum *et al.*, 2015; Chen *et al.*, 2018; Kokkoris *et al.*, 2019; Zhang *et al.*, 2019).

Many benefits have already been reported due to the use of AMs in the floriculture sector. The AMs can provide better robustness and host quality, greater durability in pot plants and less pollutants present in floral tissue and other edible organs (Sun *et al.*, 2021). In studies de Gonçalves *et al.* (2019a), irrigation and mycorrhizal inoculation effects interact in the production of total flavonoids and anthocyanins of Garden pansy, cultivar Majestic Giants II Rosalyn. The best results for total flavonoids were observed when, in the presence of mycorrhizae, 100% of the evaporated water was replaced. For total anthocyanins, better results were observed when, in the absence of mycorrhizae, 120% of the evaporated water was replaced. Saini *et al.* (2019) observed early flowering in the combination of *Funnelliformis mosseae*, *Acaulospora laevis* and *Pseudomonas fluorescens*, and also better results for floral head size, flower fresh and dry mass, total chlorophyll, carotene and phosphorus content in plants of *Gazania rigens*.

Studies on the management of garden pansy edible flowers production are still scarce. Adequate management of natural resources, especially water, as well as the use of biotechnology to reduce the quantity of chemical inputs, are relevant factors in the search for more efficient production systems.

In this study, it was considered the hypothesis that the factors water supply level and presence of arbuscular mycorrhizae jointly influence the plant growth and the crop yield edible flowers. The goal was to evaluate the growth and production of edible flowers of garden pansy plants (*Viola* × *wittrockiana* Gams.) cultivar Majestic Giants II Rosalyn, under different irrigation levels (120%, 100%, 80%, 60% and 40% of water evaporation replacement) and presence or absence of mycorrhiza, in greenhouse.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at the Federal University of São João del-Rei (UFSJ), in the municipality of Sete Lagoas, Minas Gerais State, Brazil (19° 28’ 32” S, 44° 11’ 44” W). The experiment started with sowing in May 2017 and ended in September 2017.

The experimental design was a randomized block with a two x five factorial scheme, with two levels of mycorrhizal inoculation (absence and presence) and five irrigation levels (120%, 100%, 80%, 60% and 40% of evaporation replacement) with six replicates. During the run of the experiment, the temperature and the relative humidity of the air were monitored, using a Digital Thermo-Hygrometer, with measurement of internal, external, maximum and minimum temperatures, and internal humidity, brand Incoterm® model 7666.02.0.00.

The sowing was done in tray of small round tubes, with a capacity of 55 cm^3, dimensions: 12.5 cm height; 3.5 cm outside diameter; 2.9 cm inside diameter; with six grooves and 1 hole of 1 cm in diameter. The substrate used in the sowing trays was composed of commercial vegetable land (brand Terra de Minas) without mineral fertilization, plus vermicompost (brand Adubos Bom Jardim), in the volumetric ratio of 3:1, previously homogenized, moistened and solarized in a solar collector for disinfection of pathogens,

through solar energy, for 24 hours at approximately 60 °C (Ghini, 2004).

The seedlings transplant was done to common black pots of polyethylene with a capacity of 3.1 L, dimensions of 17 cm of height, 23 cm of diameter in the mouth and 14.5 cm of diameter in the base (mark Tetraplast), lined with drainage blanket and placed on plates. Two plants were transplanted per pot, constituting the experimental units. The substrate used to fill the pots was composed of a typical soil of the biome Cerrado, homogenized with sand and organic matter, in the volumetric ratio of 2:1.5:0.5. Prior to transplanting, the substrate was moistened and solarized in a solar collector for disinfection of pathogens, through solar energy, for 24 hours at approximately 60 °C (Ghini, 2004). To fill the pots, the average mass of 2,255.45 g of substrate was used, with a standard deviation of 0.019 g. The substrate was submitted to physicochemical analysis in the laboratory LABORSOLO (Paraná, Brazil) (Table 1).

Table 1: Physical-chemical characteristics of macro and micronutrients of the substrate used in the production of garden pansy (*V. wittrockiana*) plants. Paraná, LABORSOLO, 2017

Analysis	*In Nature*	Dry Base (65 °C)
pH in $CaCl_2$ 0.01m	7.17	
Electric conductivity (µS/cm)	$1.865x10^3$	
Humidity lost at 65 °C (%)	24.08	
WHC - Water holding capacity (%)	10.07	13.26
CEC - Cation exchange capacity ($mmol_c\ kg^{-1}$)	136.00	179.14
Total phosphorus - P ($mg\ kg^{-1}$)	1.99	2.62
Total potassium - K^+ ($mg\ kg^{-1}$)	243.20	320.34
Total calcium - Ca^{++} ($mg\ kg^{-1}$)	175.80	231.56
Total magnesium - Mg^{++} ($mg\ kg^{-1}$)	64.58	85.06
Total sulfur - S ($mg\ kg^{-1}$)	48.32	63.65
Total copper - Cu^{++} ($mg\ kg^{-1}$)	0.02	0.03
Total iron - Fe^{++} ($mg\ kg^{-1}$)	1.03	1.36
Total manganese - Mn^{++} ($mg\ kg^{-1}$)	0.00	0.00
Total zinc - Zn^{++} ($mg\ kg^{-1}$)	0.01	0.01

The seeds used were garden pansy (*Viola* × *wittrockiana* Gams) cultivar Majestic Giants II Rosalyn, F1 generation, germination 93%, physical purity 99.9%, category S2 hybrid, lot 99.173, origin Japan, commercially acquired from the company Sakata®. Three seeds per cell were sown. The agronomic germination started at nine days after sowing (DAS) and at 21 DAS the thinning of the seedlings was performed. Deformed and low development seedlings were removed, leaving only one per tube. At 52 DAS, transplantation was performed for the definitive pots, with 2 plants per pot.

The soil inoculum, containing fungus *Claroideoglomus etunicatum* CNPMS09 (W.N. Becker & Gerd.) C. Walker & A. Schüßler, was obtained from the Nucleus of Applied Biology (NBA) of Embrapa Milho e Sorgo. This research is registered in the SISGEN under the number A894B2E for the access to Brazilian genetic patrimony - fungus - *C. etunicatum*, in compliance with Law nº 13,123/2015 and its regulations.

The inoculant based on inoculum soil was added to the substrate of the sowing trays in the proportion of 20% of the substrate volume, according to treatments, on the same day of sowing. To equilibrate the microbiota of the substrate of the seed trays, 1 mL of the inoculum suspension filtrate was added to each tube.

The evaluation of the mycorrhizal colonization rate and the quantification of spore density was performed at the end of the experiment. These analyzes were carried out at the

Microbial Molecular Ecology Laboratory of the Applied Biology Center, Embrapa Milho e Sorgo. The mycorrhizal colonization rate was estimated using the magnified grid-line intersection method (Giovanetti & Mosse, 1980). The structures that characterize the mycorrhizal association, such as hyphae, arborescence, and vesicles, were quantified in fragments of the pansy roots using a stereomicroscope model Zeiss SV11. The number of arbuscular mycorrhizal fungal spores was determined in 50 g of substrate sample collected from pots.

Irrigation was done using the water provided by the municipal water supply service. During the seedling production period and during the establishment period, stipulated as up to eigth days after transplanting (DAT), it was irrigated to fill 100% of the water retention capacity of the substrate. At 60 DAS (9 DAT) the application of different levels of irrigation was started according to the measurement of the water evaporation from the pots with uncultivated soils (monitoring pots) by using the weighing method. Then, daily manual irrigation was performed, applying 120%, 100%, 80%, 60% and 40% of the evaporation volume.

The evaporation volume was measured in three monitoring pots, which were weighed daily in an analytical balance with a capacity of 5 kg and an accuracy of 0.01 g. The monitoring pots, filled with substrate up to about 5 cm below the border, were previously saturated by capillarity. The upper faces of the monitoring pots were covered with plastic film to prevent evaporation during the period of saturation and subsequent monitoring of the decreasing drainage rate. In this period, the pots were removed from the tray and placed suspended to allow percolation of excess water. After 24 hours, when no percolation drip occurred in an interval of approximately 30 minutes, it was considered that the water holding capacity (pot capacity) had been reached. Then, the plastic films placed on the upper surface of the monitoring pots were removed, allowing the evaporation process.

The loss of water by evaporation in the monitoring pots was determined by means of the water balance equation, according to the expression:

$$Ev = \frac{M_i - M_{i+1}}{\rho_a} - D \qquad (1)$$

Where *Ev* is the evaporation occurring in the monitoring pot at a time interval (mL); M_i is the total mass of the monitoring pot at the beginning of the interval (g); *i* is the index representing the instant considered for the balance; M_{i+1} is the total mass of the monitoring pot at the end of the interval (g); *D* is the drainage (percolation), which eventually occurred in the time interval (cm^3) and ρ_a is the water density (1 g cm^{-3}). The volume of water lost by evaporation in monitoring pots was refilled daily to reach the pot capacity. Percolation events were recorded.

The variables height (H), number of leaves per plant (NL), base diameter (BD), shoot fresh weight (SFW), shoot dry weight (SDW), leaf area (LA), root fresh weight (RFW), root dry weight (RDW), root to shoot fresh weight ratio (RSFWR), and root to shoot dry weight ratio (RSDWR) were evaluated at the end of the experiment (115 DAS. Digitally recorded leaf images were used to calculate the total leaf area (cm^2) using the ImageJ® software. Shoot fresh and dry weight were determined by weighing in a precision analytical balance (0.0001 g). The height (H) and the base diameter (BD) were measured with a digital caliper. The number of leaves per plant (NL) were measured by manual counting. The dry matter was determined after drying in an oven with forced air circulation, for 72 hours at 70 ± 5 °C, or until it reached constant weight (Lopes & Lima, 2015).

Flowers were collected totally open weekly in the early morning to evaluate the production. The parameter product of floral dimensions, here proposed as the product between the width and height dimensions of flowers, and fresh and dry weight of the flowers were measured during the flowering start period until the final period of the experiment, which occurred between 76 and 111 DAS.

Experiment was conducted in a two x five factorial, completely randomized block design, with six replicates, at the 5% level of significance. A block design was used due to the staggering of the experiment. Normality and homoscedasticity assumptions were verified by applying Lilliefors and Levene tests, respectively. When variables studied did not meet the assumptions of the statistical model, a Box Cox transformation (Box & Cox, 1964) was applied. Transformed variables were again verified to check the assumptions of the statistical model. The analysis of variance was then performed.

When a significant interaction between the factors studied was observed, the unfolding was performed. The regression analysis at the 5% level of significance was

performed for the quantitative factor (irrigation level) when a significant difference was identified by the F-test. The ExpDes.pt package was used in the software R (R Development Core Team, 2021).

RESULTS AND DISCUSSIONS

The means of maximum, minimum and mean daily temperatures during the execution period were 33.2 °C, 13.8 °C and 23.5 °C, respectively. The means of maximum, minimum and mean daily air relative humidity were 81.1%, 29% and 55%, respectively. The physical-chemical analysis of the substrate used for garden pansy plants production presented low phosphorus content, which was already expected due to the type of soil used (Table 1).

The results of the analysis of variance are presented in tables 2 and 3. For the roots of the plants, the colonization rate presented a mean value of 32% of colonized root fragments for the plants that received the inoculant and 0% of colonization for the plants that did not receive inoculant. A mean of 224 spores per 50 g of substrate was quantified for the pots that received inoculant material. Spores were found only in the treatment that received mycorrhizal inoculation plus 120% of water evaporation replacement (Figure 1).

Table 2: ANOVA the rate of colonization (RC); height (H); number of leaves (NL); shoot fresh weight (SFW); shoot dry weight (SDW); leaf area (LA); base diameter (BD); root fresh weight (RFW); root dry weight (RDW); root to shoot fresh weight ratio (RSFWR); root to shoot dry weight ratio (RSDWR) of garden pansy (V. wittrockiana) plants, produced under greenhouse conditions under a mycorrhizal inoculation (M) factor and irrigation factor (I) at 115 DAS. Sete Lagoas, UFSJ, 2017

		MS									
F.V	G.L	RC (%)	H (cm)	NL	SFW $(g)^{0.14}$	SDW (g)	LA $(cm^2)^{0.30}$	BD (mm)	RFW (g)	RDW (g)	RSFWR	RSDWR
Blocos	5	128.90	5.1036	510.50	0.004	1.010	0.222	0.140	7.022	0.061	0.021	0.008
Mycorrhiza	1	14978.40	3.9578	851.30	0.001	0.015	0.473	0.435*	44.582*	0.195*	0.367*	0.058*
Irrigation	4	1085.40	28.9085*	13615.60*	0.220*	8.011*	9.453*	1.736*	55.507*	0.226*	0.092*	0.020*
M*I	4	1085.40*	1.6013	438.40	0.001	0.078	0.092	0.213	2.908	0.019	0.015	0.005
Erro	45	52.70	1.5752	497.50	0.004	0.461	0.277	0.101	4.762	0.038	0.026	0.006
C.V (%)		45.93	10.92	27.11	4.73	31.95	9.66	11.98	39.66	39.35	36.00	30.64

SFW and LA Cox Box transformation ($\lambda = 0.14$ and $\lambda = 0.30$, respectively). (*) Significant effect by the F test at the 5% of significance.

Table 3: ANOVA the product of floral dimensions (PFD), flower fresh weight (FFW), flower dry weight (FDW) and flower production (PROD) of garden pansy (*V. wittrockiana*) plants, produced under greenhouse conditions under a mycorrhizal inoculation (M) factor and irrigation factor (I) at 115 DAS. Sete Lagoas, UFSJ, 2017

		MS			
F.V	G.L	PFD (cm^2)	FFW $(g\ plant^{-1})$	FDW $(g\ plant^{-1})^{0.1}$	PROD $(flowers\ plant^{-1})$
Blocos	5	28.89	9.784	0.0149	6.54
Mycorrhiza	1	104.57	2.429	0.0030	2.60
Irrigation	4	1029.99*	121.905*	0.0766*	135.40*
M*I	4	10.57	3.144	0.0024	2.68
Erro	45	43.84	4.285	0.0013	8.97
C.V (%)		13.17	35.23	3.67	37.94

FDW Cox Box transformation ($\lambda = 0.1$). (*) Significant effect by the F test at the 5% of significance.

In relation to irrigation, the rate of colonization (RC) increased as the water availability increased in the pots. With a quadratic tendency, the lowest colonization rate occurred with the replacement of 42.44% of the evaporated water (Figure 2).

The absence of mycorrhizal colonization in treatments that did not receive inoculant indicated that the method of exposition of the substrate to the solar energy was effective in the elimination of propagules of native fungi. However, the rate of colonization found in the plants (32%) receiving inoculant is different from those reported in the literature. Zubek *et al.* (2015) found a colonization rate above 80% in *Viola tricolor*, using a mix of *Rhizophagus irregulares* and *Funneliformis mosseae*.

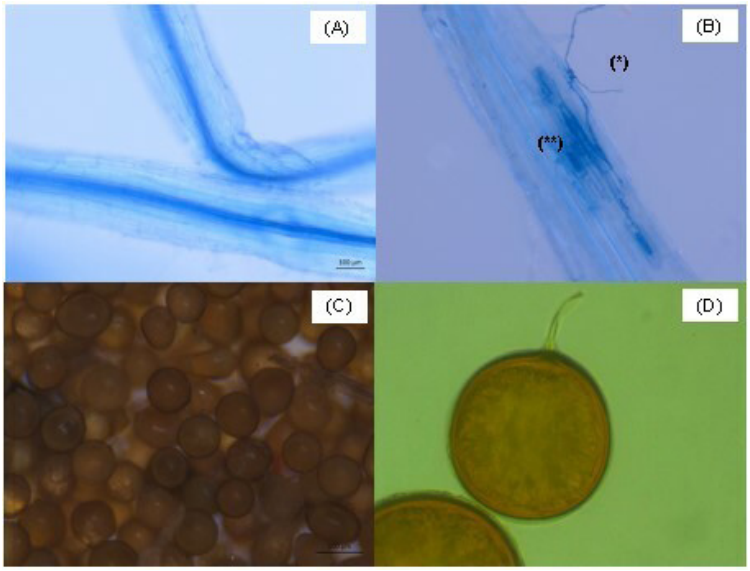

Figure 1: Root fragments free of mycorrhizal colonization (A); unit of mycorrhizal infection with presence of hyphopodia (*) and arbuscules (**) (B); spores (C. etunicatum) extracted from the substrate of the growing pots (C, D) of garden pansy (V. wittrockiana) plants. Sete Lagoas, EMBRAPA, 2017.

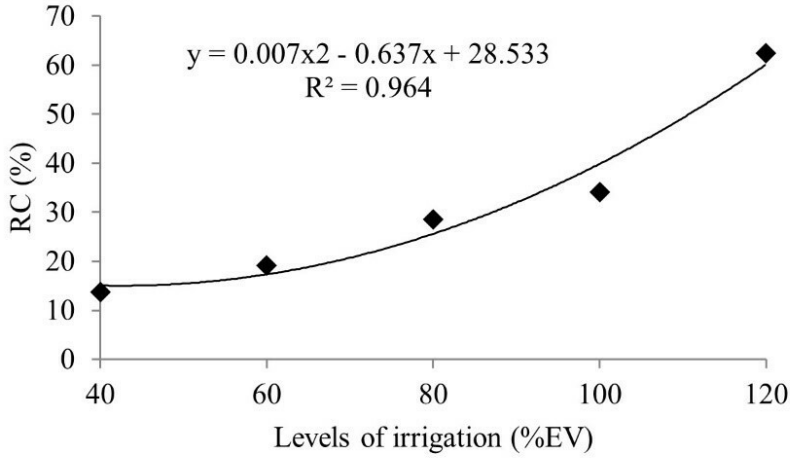

Figure 2: Rate of colonization (RC) in the root system of garden pansy (*V. wittrockiana*) plants submitted to different levels of irrigation, during production in a greenhouse. Sete Lagoas, UFSJ, 2017.

The relatively low values of the colonization rate are probably due to a low symbiotic responsiveness of the host plant to the fungus used. Symbiotic responsiveness varies in relation to host plant species, mycorrhizal fungus species and environmental conditions. In this way, it presents different values for each situation (Moreira & Siqueira, 2006; Smith & Read, 2008). On the other hand, the low colonization rate may indicate that the substrate used did not present adequate conditions for the development of mycorrhization, possibly due to the pH in the alkaline range allied to the low availability of nutrients (Table 1). It should be noted that the strain CNPMS09 was isolated from slightly acid soil and with built fertility.

The higher water availability favored higher rates of mycorrhizal colonization (Figure 2), since the mycorrhizae present better development near to the field or pot capacity, as it happens for vegetal (Moreira & Siqueira, 2006). The presence of spores in the treatment that received the highest replacement of water evaporation suggests that the fungus found conditions favorable to sporulation only in this treatment (Smith & Read, 2008), for which the highest colonization rate was found and probably the highest fungal biomass to support sporulation occurred.

The variations between the water replacement volume values of the three monitoring pots were negligible, which indicates the consistency of the method used to quantify the evaporation. The maximum, medium and minimum values of water replacement in the monitoring pots were 145.8; 82.4 and 39.4 mL, respectively. During the experiment period, there was a need for adjustments of the pot capacity due to the loss of water retention capacity. The mass reduction for the pot capacity was, on average, 183 g. The mean daily volume of water applied in the experimental units according to irrigation levels equivalent to 120%, 100%, 80%, 60% and 40% of evaporation (EV) during the experiment was 102, 85, 68, 51 and 34 mL, respectively.

There was percolation in the pots that received 120% and 100% replacement of the water evaporation. Then, an adjustment procedure was performed on the pot capacity reference. The adjustment was made by discounting the excess percolation depth observed at the 100% evaporation level. The largest percolated water volume occurred in the initial period of vegetative growth. With the growth of the plants and adjustment of the water retention capacity reference in the monitoring pots, there was a reduction in percolated volume in the experimental units that received the 120% level and there was no more percolation in the experimental units that received the 100% replenishment level of the evaporation of water, indicating that the added volume of water was consumed almost entirely in the evapotranspiration process.

The reduction of the water retention capacity in the monitoring pots was possibly due to the degradation of the organic matter present in the substrate. Fresh bovine manure presents readily decomposable material, such as proteins, starch and cellulose. These chemically unstable substances are easily attacked by decomposers microorganisms, which leads to the process of degradation or mineralization of the organic matter and to the volume reduction (Moreira & Siqueira, 2006). Allied to this, it is possible that preferential paths for water infiltration through the substrate of the monitoring pots may have arisen due the degradation of the organic matter and accommodation of the material.

The growth of garden pansy plants was analyzed at the end of the experiment period (115 DAS). For the observed variables height (H), number of leaves per plant (NL), base diameter (BD), shoot fresh weight (SFW), shoot dry weight (SDW), leaf area (LA), root fresh weight (RFW), root dry weight (RDW), root to shoot fresh weight ratio (RSFWR), and root to shoot dry weight ratio (RSDWR) there was no significant effect of the interaction between mycorrhizal inoculation factors and irrigation levels by the F test at the 5% of significance. Therefore, the factors were evaluated separately for these variables.

The mycorrhizal inoculation factor showed a significant difference in the root system variables, root to shoot ratio and base diameter (RFW, RDW, RSFWR, RSDWR and BD). The other studied variables of the aerial part showed no difference between the levels of the mycorrhizal inoculation factor (Table 4).

In the final growth analysis of garden pansy plants, the increase observed in the root-shoot ratio (Table 4) reinforces the hypothesis that the substrate had low levels of available nutrients. In this condition, the plant displaces photoassimilates into the root system with the aim of increasing the capitation of nutrients. In *Azalea* the increase in nitrogen (N) fertilization rate significantly promoted shoot growth, and low N and phosphorus (P) fertilization promoted root growth and nutrient acquisition efficiency (Ristvey *et al.,* 2007).

Higher mass values found in roots colonized with AMs have also been observed in studies with *Gazania splendens, Dimorphoteca sinuata* (Püschel *et al.*, 2014) and *Calendula officinalis* (Heitor *et al.*, 2016). This effect can

be explained by the presence of intra-root mycelia, whose biomass represents up to 20% of the weight of the roots (Smith & Read, 2008).

Non-significant effects of mycorrhization on the aerial part of inoculated plants have also been reported in the production of *Sanvitalia procumbens, Impatiens hawkerii* (Püschel *et al.*, 2014), *Viola. tricolor* (Zubek *et al.*, 2015), *Tagetes patula* and *Salvia splendens* (Janowska & Andrzejak, 2017). This fact may have occurred due to the low symbiotic efficiency, which varies according to the different species of AMs, host plant and environmental conditions (Moreira & Siqueira, 2006) and, in this way, did not provide benefits to the aerial part of the garden pansy even with the colonization effected.

Table 4: Mean height (H); number of leaves (NL); shoot fresh weight (SFW); shoot dry weight (SDW); leaf area (LA); base diameter (BD); root fresh weight (RFW); root dry weight (RDW); root to shoot fresh weight ratio (RSFWR); root to shoot dry weight ratio (RSDWR) of garden pansy (*V. wittrockiana*) plants, produced under greenhouse conditions under a mycorrhizal inoculation factor: without mycorrhizal inoculation (NM) and with mycorrhizal inoculation (M) at 115 DAS. Sete Lagoas, UFSJ, 2017

Micorrhizal inoculation	H (cm)	NL	SFW $(g)^{0.14}$	SDW (g)	LA $(cm^2)^{0.30}$	BD (mm)	RFW (g)	RDW (g)	RSFWR	RSDWR
NM	11.84 a	79 a	1.40 a	2.17 a	5.35 a	2.57 b	4.64 b	0.44 b	0.37 b	0.21 b
M	11.24 a	86 a	1.41 a	2.11 a	5.54 a	2.74 a	6.36 a	0.55 a	0.53 a	0.28 a

Means followed by the same letter in the column do not differ by the F test at the 5% level of significance. SFW and LA Cox Box transformation (λ = 0.14 and λ = 0.30, respectively).

In addition, the low content of P and other nutrients (Table 1) on the substrate was possibly not enough to stimulate the action of mycorrhizae. In soils with very low fertility, such as typical Cerrado soils, which were used for substrate composition, the addition of small amounts of P may favor the effect of inoculation on plant growth (Moreira & Siqueira, 2006).

However, positive results in the growth and development of ornamental plants inoculated with AMs are reported in works with *Viburnum tinus* (Gómez-Bellot *et al.*, 2015); *Cyclamen purpurascens* (Rydlová *et al.*, 2015); *Sinningia speciosa* (Janowska *et al.*, 2016) *Rudbeckia laciniata* and *Solidago gigantea* (Majewska *et al.*, 2017); *Calendula officinalis* (Kheyri *et al.*, 2022).

Significant differences were detected on the variables of plant growth at the end of the experiment in response to the irrigation levels. A linear effect was observed for the variables SDW, RFW, H and NL, indicating that for each 1% of water evaporation, there was growth of 0.025 g; 0.064 g; 0.046 cm and 1.018 units, respectively (Figure 3).

For the variables SFW, RDW, BD and LA, quadratic behavior was observed, with points of maximum vegetative growth in water replenishment of 107.1%; 96.8%; 104.8% and 116.2% EV (Figure 4).

The reduction of plant biomass verified in garden pansy plants in response to low irrigation levels (Figure 3 and Figure 4) have also been observed by Cirillo *et al.* (2017) in *Bougainvillea* production, in which, in addition to the decrease in plant biomass, morpho-physiological changes in response to the irrigation deficit also occurred. The same situation was observed Ugolini *et al.* (2015) when evaluating the production of *Viburnum opulus* and *Photinia* × *fraseri* under water deficit, outdoors and in a greenhouse, that found higher vegetative growth for treatment in which 100% of the crop evapotranspiration determined the water replacement quantity. Elansary *et al.* (2016), in the production of *Spiraea nipponica* and *Pittosporum eugenioides*, and Álvarez & Sánchez-Blanco (2013; 2015), in the production of *Callistemon citrinus* and *Callistemon laevis*, also obtained similar responses in relation to low water availability.

The gain of plant biomass is closely linked to the water availability to the crops, since cell growth only occurs due to turgor tension, exerted by the water inside, guaranteeing the expansion of the cell wall, its growth in extension and the cell division. In addition, a number of metabolic processes are affected by water deficiency, such as stomatal closure, nutrient absorption, and the metabolism of proteins

and amino acids, which impedes the correct cellular functioning and, therefore, the vegetal growth (Taiz & Zeiger, 2013; Lopes & Lima, 2015).

A linear downward effect was observed for RSFWR and RSDWR variables, indicating that for each 1% of water evaporation replacement, there was a decrease of 0.003 g and 0.001 g in the ratio of fresh and dry biomass between root and shoot, respectively (Figure 5).

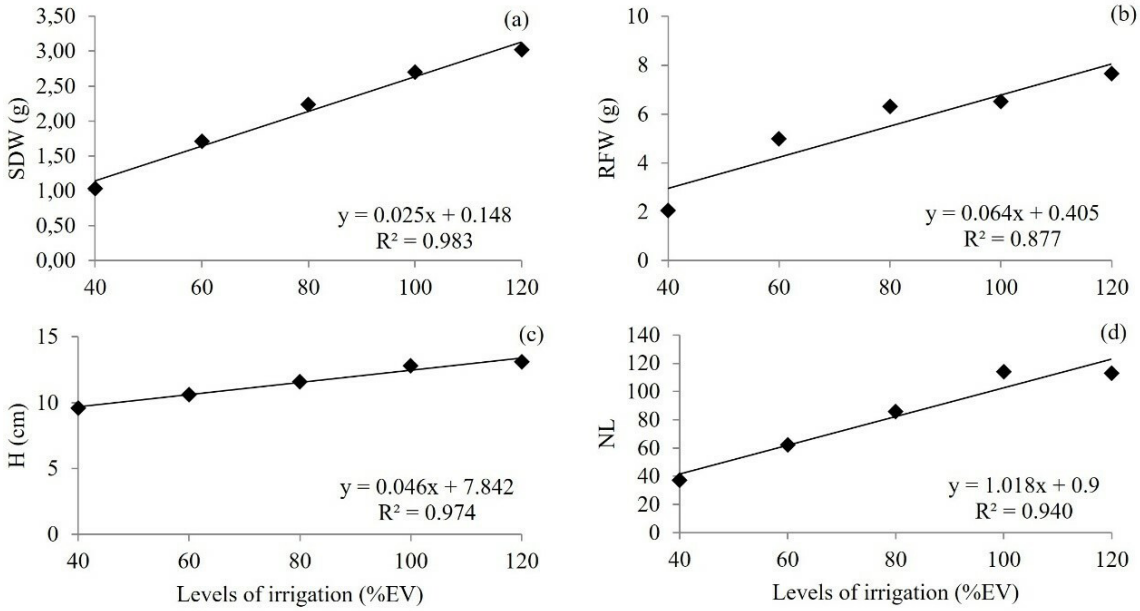

Figure 3: Evaluation of shoot dry weight (SDW); root fresh weight (RFW); height (H) and number of leaves/plant (NL) of garden pansy (*V. wittrockiana*) plants versus levels of irrigation at 115 DAS. Sete Lagoas, UFSJ, 2017.

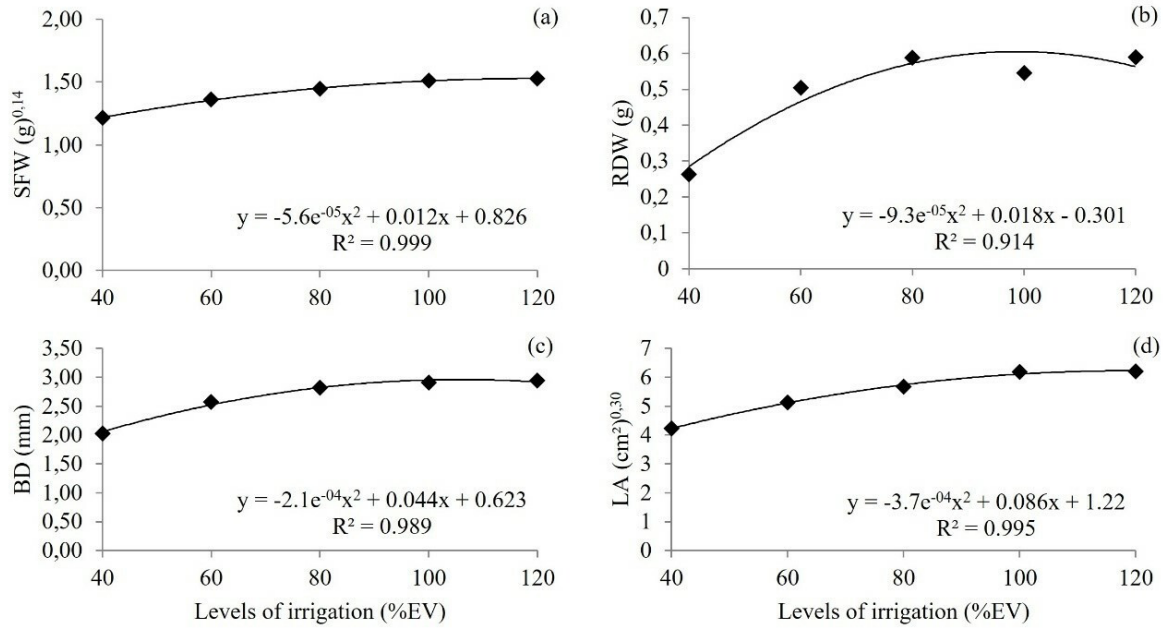

Figure 4: Average of shoot fresh weight (SFW); root dry weight (RDW); base diameter (BD) and leaf area (LA) of garden pansy (*V. wittrockiana*) plants versus levels of irrigation at 115 DAS (SFW and LA Box Cox transformation, $\lambda = 0.14$ and $\lambda = 0.30$, respectively). Sete Lagoas, UFSJ, 2017.

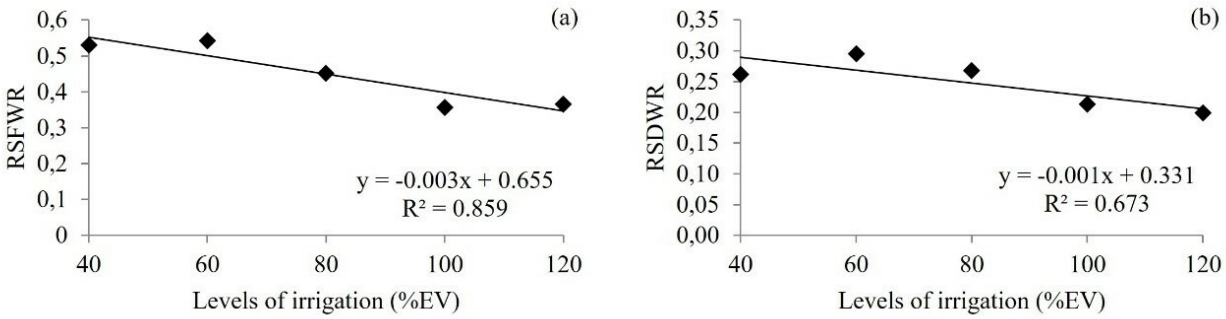

Figure 5: Evaluation of the root to shoot fresh weight ratio (RSFWR) and root to shoot dry weight ratio (RSDWR) of garden pansy (*V. wittrockiana*) plants versus levels of irrigation. Sete Lagoas, UFSJ, 2017.

One of the plant strategies to mitigate the possible damages caused by the lack of water is the root growth, allowing greater exploitation of the soil in search of water (Lopes & Lima, 2015). This action was observed in garden pansy plants, which presented higher values of RSFWR and RSDWR when they had less water availability (Figure 5-a, b, respectively). The same occurred in studies with *Callistemon citrinus* (Álvarez & Sánchez-Blanco, 2013) and *Viburnum opulus* and *Photinia x fraseri* (Ugolini *et al.*, 2015).

The flower production, with the variables product of floral dimensions (PFD), flower fresh weight (FFW), flower dry weight (FDW) and production (PROD), evaluated at the end of the experiment (115 DAS), did not present a significant interaction, by the F test at the level of 5% of significance, between the factors mycorrhizal inoculation and irrigation levels. Therefore, the factors were evaluated separately.

Regarding the mycorrhizal inoculation factor, there was no significant difference between their levels in the flowering variables (Table 5). However, significant differences were observed in the flowering variables of the garden pansy plants for the irrigation levels. The performance presented in the reproductive stage of the pansy plants reflects what occurred in the vegetative growth phase.

The observed results show increases in PFD according to the greater availability of water for the plants, following a linear tendency with an increase of 0.289 cm² in each 1% of the EV that was restored (Figure 6-a). For the variables of PROD, FFW and FDW, quadratic tendencies were observed, with maximum production and floral growth points at 98.9% (10.8 flowers plant^{-1}), 111.8% (8.1 g plant^{-1}), and 103.8% (1.1 g plant^{-1}) EV, respectively (Figure 6-b, c, d).

The lack of influence of mycorrhizal inoculation on flower production has already been reported in studies with *Gazania splendens, Capsicum annuum, Sanvitalia procumbens*, *Pelargonium peltatum* and *P. zonale* (Püschel *et al.*, 2014). Probably, the low responsivity of garden pansy (*V. wittrockiana*) to the fungus *C. etunicatum* in relation to plant growth was reflected in the reproductive phase.

Table 5: Evaluation of product of floral dimensions (PFD), flower fresh weight (FFW), flower dry weight (FDW) and flower production (PROD) of garden pansy (*V. wittrockiana*) plants in response to the mycorrhizal inoculation factor: without mycorrhiza (NM) and with mycorrhiza (M), produced in a greenhouse (FDW transformation Box Cox with λ = 0.1). Sete Lagoas, UFSJ, 2017

Mycorrhizal Inoculation	PFD (cm²)	FFW (g plant^{-1})	FDW (g plant^{-1})^{0,1}	PROD (flowers plant^{-1})
NM	51,61 a	6,08 a	0,98 a	8,10 a
M	48,97 a	5,67 a	0,96 a	7,68 a

Means followed by the same letter in the column do not differ by the F test at the 5% level of significance. SFW and LA Cox Box transformation (λ = 0.1).

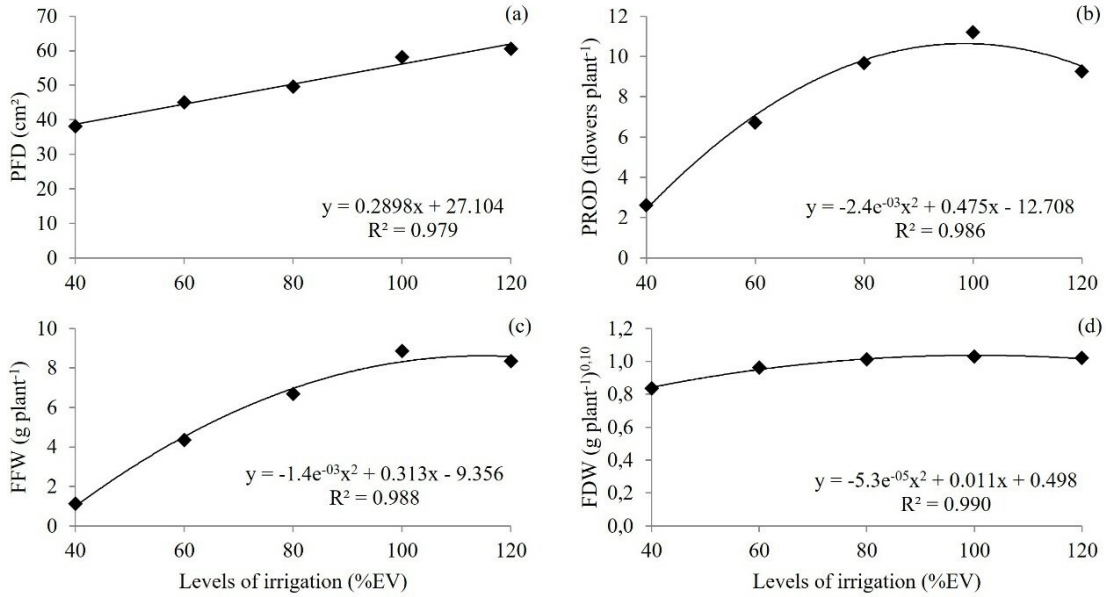

Figure 6: Evaluation of product of floral dimensions (PFD), flower production (PROD), flower fresh weight (FFW) and flower dry weight (FDW) of garden pansy (*V. wittrockiana*) plants versus levels of irrigation (FFW transformation Box Cox with λ = 0.10). Sete Lagoas, UFSJ, 2017.

On the other hand, irrigation positively influenced the production of garden pansy flowers (Figure 6). Similar results were found by Aleman & Marques (2016) that observed increase in the dry matter production of flower buds of *Chamomilla recutita* for higher irrigation levels.

Plants cultivated under suitable water availability tend to present higher nutrient absorption capacity and photosynthetic rate, which enhances the vegetative and reproductive growth (Taiz & Zeiger, 2013; Lopes & Lima, 2015). Under the conditions of the experiment, higher water availability resulted in greater average values of vegetative growth and of the flowering variables studied, indicating the relevance of irrigation management.

Here we showed that irrigation is fundamental for the production of garden pansy flowers. At the commercial level, cost efficient irrigation systems can be implemented and they might guarantee a stable production of garden pansy flowers. The results showed no effect of the mycorrhizal inoculation on the production of garden pansy flowers. However, they reinforce the need for additional research applied to the use of different strains of AM fungi and other microbes (Saini *et al.*, 2019), seeking to increase the efficiency of water and nutrient extraction and, consequently, to achieve higher crop yields in floriculture.

CONCLUSIONS

The factors mycorrhizal inoculation and irrigation act independently in vegetative growth and flower production of garden pansy (*V. wittrockiana*) plants, cultivar Majestic Giants II Rosalyn. There were no significant increases in the growth and production of flowers in response to inoculation with mycorrhizal fungi *Claroideoglomus etunicatum* CNPMS09, even obtaining effective colonization and reproduction of the fungus. Better growth and yield results were obtained when irrigated with 100% replenishment volume of water evaporation, which indicates water stress for lower irrigation depths.

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