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Full Length Research Paper

Phytomass and flavonoid production in different organs and phenological stages of *Passiflora alata* Dryander

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The present study evaluated the dry matter mass production in different *Passiflora alata* Dryander organs and phenological stages, as well as their correlation with phytochemical quality in relation to the flavonoids vitexin, isoquercetin, quercetin and rutin. Two experiments were carried out: Experiment 1, effect of different organs (flower, leaves, fruits and branches), and Experiment 2, effect of phenological stages (pre-flowering, flowering, fruiting and post-fruiting). Both experimental designs were in randomized blocks with five and six replicates, respectively. The highest flavonoid concentration and production were detected in leaves. On the other hand, branches and fruits did not have significant rutin and isoquercetin content. The largest leaf dry matter mass and flavonoid production was observed in the post-fruiting stage, that is, at 300 days after transplanting (DAT). Flower formation was a strong deleterious competitor to leaf and branch dry matter mass production, as well as to biosynthesis and accumulation of the evaluated flavonoids. In addition to obtain *P. alata* - based drugs of high pharmaceutical quality, further studies on flowering management, as well as agronomical research, are needed.

Key words: Sweet passion fruit, phytochemical quality, medicinal plant, management.

INTRODUCTION

Passiflora alata Dryander is commonly known as sweet passion fruit. Several *Passiflora* species are native to Brazil and their uses vary from alimentary to medicinal (Hoehne, 1946). Besides its economic importance in fruitculture, *P. alata* is one of the most important plants in the phytotherapeutic market. This species is also included in the Brazilian Pharmacopeia monograph (1977). Its shoot is used in the preparation of phytomedicines (ANVISA, 2003) for insomnia and menopause-related stress, or diuretic, antifungal, anti-inflammatory, anxiolytic and sedative drugs (Freitas, 1987; Junior et al., 2000; Montanher et al., 2007; Pelegrini et al., 2006; Arnal-Schnebelen and Goetz, 2007; Reginatto et al., 2006; Rojas et al., 2006). In addition, preclinical tests confirmed

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that ethanolic extract and juice from *Passiflora edulis* are nontoxic and have anti-hypertensive action (Rojas et al., 2006), as well as protective effect against lipid peroxidation and tissue necrosis, including hepato and cardioprotective potential associated with blood pressure reduction (Rudnicki et al., 2007).

Species of *Passiflora* genus present C-glycoside flavonoids, such as vitexin, orientin, isoquercetin, isoorientin and rutin,as well as indolic alkaloids applicable to the central nervous system (Pereira and Vilegas, 2000; Dhawan et al., 2004; Zuanazzi, 2001). According to Muller et al. (2005), vitexin and isoquercetin also have anti-inflammatory, antioxidant, hypotensive, anti-spasmodic, antimicrobial, and radio-protective effects.

In general, quantitative and qualitative phytochemical aspects significantly vary with the genetic access, the phenological stage, the nutritional and ecophysiological states, the organ and its part and ontogenetic stage, besides the distribution of the produced phytomass and special metabolites among plant organs (Gobbo-Neto and Lopes, 2007; Guerreiro 2006; Marchese, 2006; Guerreiro, 2006; Marchese et al., 2005; Mattana, 2005; Marchese and Figueira, 2005; Muller et al., 2005; Dhawan et al., 2004; Taiz and Zeiger, 2004; Figueiredo, 2003; Larcher, 2000; Reis and Mariot, 2001; Castro et al., 2000; Marchese, 1999; Di Stasi, 1996; Gotlieb et al., 1996; Correa Jr. et al., 1994; Menghini et al., 1993). Zuanazzi (2001) alerted to a possible qualitative and quantitative variation in flavonoids among different *P. alata* organs and stages. Isoquercetin production in *P. alata* leaves is strongly influenced by seasonality and is larger in the summer than in the winter (Muller et al., 2005). Thus, there are natural variations in *P. alata*, which are responsible for the differences in the pharmacological quality of the plant medicine. Therefore, there is a need to search for technical information on the quality of raw material for plant drugs during the several phenological stages of *P. alata*, as well as to understand how such stages affect the production of flavonoids which are decisive among other factors, for quality standardization and control of the plant drug and its byproducts (Marchese and Figueira, 2005, DI Stasi, 1996). Thus, the aim of the present work was to evaluate dry matter mass production in different *P. alata* Dryander organs and phenological stages and their correlation with phytochemical quality considering the flavonoids vitexin, isoquercetin, quercetin and rutin.

MATERIALS AND METHODS

Plants were grown under organic production system in the experimental field from Department of Plant Production, Horticulture Sector, Agronomical Sciences College, São Paulo State University–UNESP, Botucatu, São Paulo State, Brazil. The soil was red latosol with base saturation adjusted to 60% and was fertilized with 4 kg m^{-2} chicken manure which had the following characteristics: C/N = 19/1; pH = 7.66; total N = 10; P_2O_5 = 9.6; K_2O = 8.8; Ca^{2+} = 24; Mg^{2+} = 11.4; S = 3.0; O.M. = 340; and Carbon = 180 g kg^{-1} associated with Na^{+} = 1880; Cu^{2+} = 120; Fe^{2+} = 21300; Mn^{2+} = 512; Zn^{2+} = 128 mg kg^{-1}. *P. alata* seedlings were produced in a nursery through sowing in plastic bags containing substrate prepared with three parts of soil, one part of cattle manure and one part of carbonized rice hulls (Kavati and Piza, 2002). Plants presenting 4 to 5 definitive leaves were transplanted. Spacing was 4 mamong rows and 2.5 mamong plants, which were cultivated in trellisusingwire. The plants underwent conduction pruning to make branches directed to both wire sides, followed byapex pruning at approximately 1.25 m to allow the formation of lateral branches (crown). Then, no additional pruning was done.

Experiment 1

Production of the flavonoids rutin, vitexin, quercetin and isoquercetin in different organs (flower, leaves, fruits and branches) of *P. alata* at 226 days after transplanting (DAT) in randomized blocks with five replicates of 10 plants each.

Experiment 2

Total phytomass production per organ, and rutin, vitexin, quercetin and isoquercetin content and production in leaves during four *P. alata* phenological stages (before flowering, during flowering, during fruiting, after fruiting) in randomized blocks with six replicates of 10 plants each.

After weighing, tissues from both experiments were dried at 40°C in a forced aeration oven and ground in Wiley-type grinder.

Flavonoids were analyzed from methanolic extract of tissue dried at 40°C. The extract was purified through the elimination of chlorophyll and other pigments with the aid of C-18 cartridge (*Strata SPE Products*). The remaining extract was subjected to complexing with aluminum chloride and identified and quantified in relation to wavelengths characteristic of quercetin (430 nm), isoquercetin (339 nm), rutin (424 nm) and vitexin (419 nm) standards using a UV-Visible spectrophotometer (HITACHI, Model: U-2000) and a standard curve previously established for each flavonoid.

Results were subjected to contrast through Scott-Knotttest and Pearson correlation analysis, both with < 5% error probability.

RESULTS AND DISCUSSION

In Experiment 1, flavonoid distribution among *P. alata* organs significantly varied. Flowers generally accumulate large quantities of flavonoids through ecological relations (Gobbo-Neto and Lopes, 2007; Taiz and Zeiger, 2004; Mann, 1994; Zuanazzi, 2001; Larcher, 2000); however, this fact was not observed in the present study, relative to the remaining organs.

The highest flavonoid content was detected in leaves (Table 1), which accumulated 81.00, 92.68, 93.00, and 85.00% of total quercetin, isoquercetin, rutin and vitexin, respectively, produced by the plant. Thus, the remaining flavonoids accumulated in flowers, fruits and branches, except for isoquercetin which was not found in fruits and branches, as well as rutin, also absent in branches. The qualitative and quantitative variation observed among *P. alata* organs agrees with the report of Zuanazzi (2001) about the existence of such variation in plant species in general.

Metabolite biosynthesis and accumulation is strictly

Table 1- Flavonoid content in *P. alata* Dryander organs.

Organ	Quercetin	Isoquercetin	Rutin	Vitexin
	mg g^{-1} dry matter mass in the organ			
Flower	0.052 c	0.238 b	0.232 b	0.211 b
Leaf	0.391 a	1.142 a	1.333 a	1.146 a
Fruit	0.020 d	0.000 c	0.069 c	0.082 b
Branch	0.093 b	0.000 c	0.000 c	0.152 b

Means followed by the same lowercase letter in the column do not differ according to Scott-Knott test ($P < 0.01$).

related to the availability of environmental resources, energy, moderate stress level and carbon residues and other substrates (Gobbo-Neto and Lopes, 2007; Gotlieb et al., 1996; Marchese and Figueira, 2005; Guerreiro, 2006, Correa Jr. et al. 1994). According to Simmonds (2003), the biosynthesis and accumulation of the studied flavonoids in the leaves is justifiable since they participate in leaf protection against herbivores and microorganisms. Also, Zuanazzi (2001) emphasized that some flavonoids act as phytoalexins during the attack of phytopathogens. In passion fruit culture, diseases and plagues are capable of making it economically impracticable due to their great complexity (Pio-Ribeiro and Mariano, 1997; Gallo et al., 2002).

The results obtained in Experiment 1 lead to the conclusion that leaves are the best sources of the evaluated flavonoids since their biosynthesis occurs in such organ and they participate in the polar transportation of auxins as well as in the vegetative growth of plants (Taiz and Zeiger, 2004; Zuanazzi, 2001). Thus, leaves act as source and drain since flavonoid biosynthesis supplies their own reserves, and their products lead to the growth of the remaining drains due to polar transportation of auxins. Considering *P. alata* industrial processing, the present results indicate that an unsuitable leaf separation from the remaining organs (Table 2) will lead to dilution of approximately 55% flavonoids. Therefore, this fact contributes to the increase in logistic and processing costs, besides the possibility of affecting the phytochemical qualitative standard of the plant medicine and its byproducts. In conclusion, the most interesting plant part from an agronomical and phytochemical point of view is the leaf to the detriment of the remaining organs, regarding phytomass productivity and quercetin, vitexin, isoquercetin and rutin synthesis by *P. alata*.

The results obtained in Experiment 2 for the effect of phenological stages on dry matter mass andvitexin, isoquercetin, quercetin and rutin production indicated that leaf dry matter mass per *P. alata* plant increased over phenological stages; thus, the largest production was detected in post-fruiting (429.818 g pl^{-1}). Although, there was a significant difference between flowering and fruiting stages, leaf dry mass production was less intense in such stages (Table 2). The largest number of leaves per plant was also observed in post-fruiting (624.024 a) and the smallest number in pre-flowering (166.258 c), with no significant differences between flowering and fruiting stages (Table 2). Thus, flowers are preponderant as drains over the remaining plant organs, based on the phytomass distribution and the organ formation in *P. alata* evaluated in the present study. The development of *P. alata* branches and, consequently, their differentiation as vegetative and reproductive alters the relation source/drain (Vasconcellos et al., 2002a, b); both branch types can occur in the same plant at the same time. According to that author, based on ^{13}Cisotype partition in *P. alata*, the major drains in branches during the vegetative stage are young leaves and stem apex, whereas flowers are the main drains in the reproductive stage, paralyzing or reducing the allocation of resources to vegetative organs.

The same pattern was observed by Marchese et al. (2005) in *Artemísia annua*; however, senescence occurred after the reproductive stage. The present results and the reports of Vasconcellos et al. (2002a, b) corroborated those by Larcher (2000), Taiz and Zeiger (2004) and Marenco and Lopes (2005). Plants showing this pattern are characterized as investor-type plants, generally herbaceous and/or annual of indeterminate growth (Larcher, 2000). The number of flowers significantly and negatively correlated with leaf number ($r = -0.40$; $P = 0.001$) and dry matter mass of leaves ($r = -0.46$; $P = 0.001$), branches ($r = -0.42$; $P = 0.001$) and fruits ($r = -0.41$; $P = 0.001$), and was preponderant, as drain, over the remaining organs.

Over the phenological stages, there was a significant and gradual increase in the leaf level (mg g^{-1}) of quercetin (0.639 a), isoquercetin (1.569 a), vitexin (1.606 a) and rutin (2.123 a). As the same pattern was observed for leaf dry matter mass, the largest production (mg pl^{-1}) of quercetin (274.65 a), isoquercetin (673.78 a), vitexin (689.63 a) and rutin (911.68 a) was obtained after fruiting (Table 3).

In general, the biosynthesis of special metabolites includes costs of energy and carbon which may compete with or be favoured by the primary metabolism, depending on its status (Gobbo-Neto and Lopes, 2007;

Table 2. Dry matter mass, organ and flavonoid production by *P. alata* Dryander in different phenological stages.

Stage	Phytomass and organs			
	Leaves	**Branches**	**Flowers**	**Fruits**
Dry matter mass (g pl $^{-1}$)				
Pre-flowering	96.837 d	67.183 d	4.985 b	0.000 c
Flowering	294.514 c	211.927 c	111.466 a	39.362 b
Fruiting	336.576 b	246.134 b	0.000 c	46.262 b
Post-fruiting	429.818 a	468.549 a	0.000 c	60.548 a
Number of organs (pl $^{-1}$)				
Pre-flowering	166.258 c	*	44.730 b	0.000 c
Flowering	398.612 b	*	81.870 a	4.630 b
Fruiting	423.043 b	*	0.000 c	7.684 a
Post-fruiting	624.024 a	*	0.000 c	9.429 a
Flavonoid content (mg g $^{-1}$ leaf dry matter mass)				
	Quercetin	**Isoquercetin**	**Vitexin**	**Rutin**
Pre-flowering	*	*	*	*
Flowering	0.388 c	1.029 c	0.905 c	1.076 c
Fruiting	0.540 b	1.405 b	1.301 b	1.494 b
Post-fruiting	0.639 a	1.569 a	1.606 a	2.123 a
Flavonoid production in leaves (mg pl^{-1})				
Pre-flowering	*	*	*	*
Flowering	114.588 c	303.665 c	267.341 c	317.636 c
Fruiting	181.482 b	472.331 b	437.304 b	502.545 b
Post-fruiting	274.653 a	673.784 a	689.629 a	911.685 a

Means followed by the same lowercase letter in the column do not differ according to Scott-Knott test ($P < 0.01$). Pre-flowering, 190 DAT; Flowering, 226 DAT; Fruiting, 254 DAT; Post-fruiting, 300 DAT. *, data not measured due to absence of applicability in the case of branch number and to the lack of sufficient material for analysis in the case of flavonoids.

Table 3. Flavonoid production by *P. alata* Dryander in different phenological stages.

Stage	Flavonoid production in the leaves (mg pl^{-1})			
	Quercetin	**Isoquercetin**	**Vitexin**	**Rutin**
Pre-flowering	*	*	*	*
Flowering	114.588 c	303.665 c	267.341 c	317.636 c
Fruiting	181.482 b	472.331 b	437.304 b	502.545 b
Post-fruiting	274.653 a	673.784 a	689.629 a	911.685 a

Means followed by the same lowercase letter in the column do not differ according to Scott-Knott test ($P < 0.01$). Pre-flowering, 190 DAT; Flowering, 226 DAT; Fruiting, 254 DAT; Post-fruiting, 300 DAT. *, data not measured due to absence of applicability in the case of branch number and to the lack of sufficient material for analysis in the case of flavonoids.

Gotlieb et al., 1996; Marchese, 1999; Marchese and Figueira, 2005). For flavonoid production, phenological stage, appropriate drains and sources, complete photosynthesis, nutrients, light, CO_2, energy and availability of the amino acid phenylalanine are essential (Mann, 1994; Taiz and Zeiger, 2004; Marchner 1995; Marschner 1995; Herbert, 1989).

According to Muller et al. (2005), in *P. alata* leaves, isovitexin is under the effect of seasonality. Thus, its content is lower in leaves collected in the winter than in those collected in the summer probably due to the lower solar energy inputduring the winter, relative to summer

under tropical conditions.

The flavonoids act in the production of vegetative and reproductive organs to participate in the balance organ source (leaves) and sink organs (fruits). This fact is justified by acting as modulators of polar transport of auxin and other hormones involved in meristematic development, apical dominance and fruit set (Taiz and Zeiger, 2004).

Flavonoids are also associated with the plant defense against herbivores (Simmonds, 2003). This author states that the higher the rutin concentration, for example in leaf tissue, the lower the attack by herbivores.

In addition, moderate stress level can trigger the biosynthesis of metabolites necessary for the physiological protection and maintenance of the primary metabolismin plants (Gotlieb et al., 1996; Marchese, 1999; Marchese and Figueira, 2005). Thus, appropriate resource supply must be associated with a sufficient photosynthetic potential in order to provide carbon and energy in excess, which are destined for special or secondary metabolism (Gotlieb et al., 1996).

After playing their roles, special metabolites are recycled to primary metabolism products, energy, and/or CO_2, similarly to the anabolic and catabolic reactions of some flavonoids (Gotlieb et al., 1996). This explains, at least in part, the qualitative and quantitative transience of metabolites in the plant and/or its organs.

However, the possibility of combinations of these conditions must be considered in agronomical management of *P. alata* for the production of bioactive molecules, in this case flavonoids. Thus, in this study, flavonoid biosynthesis was probably favoured by the maintenance of sources, that is, photosynthetically active tissues (leaves) (Table 2). This hypothesis is reinforced by the detection of a significant and positive correlation of leaf dry matter mass and number, respectively, with the level and production of quercetin (r = 0.94; P = 0.001; r = 0.89; P = 0.0001), (r = 0.95; P = 0.001; r = 0.91; P = 0.0001); isoquercetin (r = 0.93; P = 0.0001; r = 0.87; P = 0.001), (r = 0.95; P = 0.001; r = 0.90; P = 0.0001); rutin (r = 0.93; P = 0.000; r = 0.91; P = 0.000), (r = 0.91; P = 0.001; r = 0.91; P = 0.0001); and vitexin (r = 0.93; P = 0.000; r = 0.88; P = 0.001), (r = 0.92; P = 0.001; r = 0.89; P = 0.0001).

Similarly to the correlations observed for leaves, flowers also showed to be preponderant drains over flavonoid biosynthesis, since the number of flowers negatively correlated with the level and production of quercetin (r = - 0.51; P = 0.0001) (r = - 0.60; P = 0.000), isoquercetin (r = - 0.48; P = 0.001) (r = - 0.59; P = 0.001), rutin (r = - 0.53; P = 0.001) (r = - 0.60; P = 0.0002), and vitexin (r = - 0.51; P = 0.0003) (r = - 0.60; P = 0.0001), respectively.

Therefore, studies on agronomical managementaimed at better vegetative development associated with pruning or hormonal management of reproductive drains, especially flowers, may favour the biosynthesis and productivity of the evaluated flavonoids and leaf phytomass.

Conclusions

Leaves are the main sources of vitexin, isoquercetin, quercetin and rutin, and contain, an average, 90% of the total flavonoids produced by the plant. However, branches and fruits are not significant sources of rutin and isoquercetin. The largest production of leaves and flavonoids occurred in the post-fruiting stage and was negatively affected by flower formation.

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