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Influence of base saturation on buckwheat grain and flavonoid production

Influência da saturação por bases na produção de grãos e de flavonoides de trigo-mourisco

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Highlights

Base saturation does not influence flavonoid production.
Dry matter yield is highest near 62.00% base saturation.
Base saturation influences grain yield.
IPR 92 is the higher-yielding cultivar.
Buckwheat is a species with great industrial potential for flavonoids.

Abstract

Fagopyrum esculentum is highly nutritious due to its proteins of high biological value and high fiber content. Its most relevant property is nonetheless its antioxidant activity, provided by the presence of flavonoids. It is an important pseudocereal in agriculture, animal production, and human food. The objective of study was to evaluate the grain yield and flavonoid production of *Fagopyrum esculentum* cultivars as a function of base saturation. The experiment was carried out in a greenhouse, in a completely randomized design with four replicates. Treatments were represented by cultivars IPR 91 and IPR 92 and five base saturation levels (9, 31, 53, 75, and 97 %), in a factorial arrangement (2 × 5). Agronomic attributes and total-flavonoid and rutin contents and production were evaluated. Subsequently, the data were subjected to statistical analysis. Base saturation does not affect flavonoid production. However, base saturation influences shoot dry matter yield (maximum at 62.0% base saturation) and grain yield (maximum at 9.00%) in IPR 92. The higher-yielding cultivar is IPR 92. This is the first scientific report of base saturation in the species.

Key words: Antioxidant activity. Biological value. *Fagopyrum esculentum.* Pseudocereal. Rutin.

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Resumo

Fagopyrum esculentum é muito nutritivo por conter proteínas com alto valor biológico e alto teor de fibras, a propriedade mais relevante dele é a atividade antioxidante, devido à presença de flavonoides. É um pseudocereal importante na agricultura, produção animal e alimentação humana. O objetivo desse trabalho foi avaliar a produção de grãos e de flavonoides em cultivares de *Fagopyrum esculentum* em função da saturação por bases. O experimento foi realizado em casa de vegetação em delineamento inteiramente casualizado, com quatro repetições. Os tratamentos foram as cultivares IPR 91 e IPR 92 e cinco saturações por bases (9, 31, 53, 75 e 97 %), em esquema fatorial (2 × 5). Foram avaliados atributos agronômicos e teor e produção de flavonoides totais e de rutina. Posteriormente os dados foram submetidos a análises estatísticas. Para a produção de flavonoides, a saturação por bases não influencia nos resultados. Porém, para a produção de matéria seca da parte aérea (produção máxima aos 62,0 % de saturação por bases) e para a produção de grãos da IPR 92 (produção máxima aos 9,00 %), a saturação por bases influencia. A cultivar de maior produção é a IPR 92. Esse é o primeiro relato científico de saturação por bases da espécie.

Palavras-chave: Atividade antioxidante. *Fagopyrum esculentum.* Pseudocereal. Rutina. Valor biológico.

Introduction

Studies on the development of plants and secondary metabolites are extremely important, particularly in species holding great nutritional and medicinal potential in national and global rise, as is the case of buckwheat (Görgen et al., 2016). Understanding how a plant behaves in given environments and different soil, humidity, and stress conditions is essential for the improvement of the species as well as to spread its cultivation and achieve higher yields. The literature has no indications of the ideal soil base saturation level for buckwheat production, and neither are there reports in this respect, especially aiming at grain and flavonoid production, considering that the species is used for both purposes.

Buckwheat grains and derivatives are gluten-free (Giménez-Bastida, Piskula, & Zieliński, 2015). In addition, compared with *Triticum* spp., buckwheat grains have a higher content of antioxidant substances, including flavonoids (Sedej et al., 2012). Buckwheat is the only grain species containing rutin (Joshi et al., 2019). Another advantage is its flour, which can be used as a source of rutin (Bai et al., 2015). Enriching buckwheat flour with the husk of its grains broadens the use of the plant and the antioxidant quality of the product (Sedej et al., 2012). Because it is a functional food, buckwheat-derived products are sold at higher prices than common-wheat products, which buckwheat is able to replace (Relva Verde, 2020).

Studies investigating flavonoid levels in plants are necessary to understand how these compounds react to different cultivation processes, assuming that secondary metabolites are plant defenses against biotic and abiotic stimuli (Zhao et al., 2014). Besides interfering with the plant's development throughout the phenological stages, nutrient availability is one of the factors that interfere with the chemical composition of secondary metabolites, mainly in annual-cycle plants. In this respect, soil base saturation is one of the essential factors for nutrients to become available to plants, and buckwheat is an aluminum-accumulator species (Horbowicz, Kowalczyk, Grzesiuk, & Mitrus, 2011; Xu et al., 2017) that can grow better in acidic soils.

Base saturation is an important factor to be taken into account to grow crops on a large scale, meet the industrial market demand, and enable the country to produce at sufficient levels. In the specific case of buckwheat, this study also contributes to the improvement of the species by determining the best growing conditions for optimal production of grains, biomass, and flavonoids. This is favorable for producers who aim to implement the crop, who will benefit from greater security and assertiveness.

Material and Methods

Experimental location

The experiment was carried out in a greenhouse at the Institute of Agricultural Sciences (ICA) at the Federal University of Minas Gerais (UFMG), located in Montes Claros - MG, Brazil (16º40'58.5" S and 43º50'25.6" W, 645.9 m above sea level). Average annual temperature and precipitation in the region are 22.7 ºC and 1029 mm, respectively (Instituto Nacional de Meteorologia [INMET], 2019). Situated in *cerrado* and *caatinga* biome areas (Instituto Brasileiro de Geografia e Estatística [IBGE], 2019), the studied region has a climate classified as a hot and semi-humid, tropical type (Aw) (Alvares et al., 2013) with well-defined seasons, a prolonged dry period, and a short rainy period. During the experiment, the average minimum and maximum relative humidity values were 65.4 and 71.6% and the average minimum and maximum temperatures were 23.1 and 24.4 ºC, respectively (INMET, 2019).

Experimental procedure and evaluations

The experiment was conducted in 5-dm³ pots filled with dystric soil (pH in water = 3.8; P = 2.0 mg dm^{-3}; Ca = 0.9 cmolc dm^{-3}; Mg = 0.5 cmolc dm^{-3}; K = 0.1 cmolc dm-³; Al = 4.0 cmolc dm^{-3}; H + Al = 15.0 cmolc dm^{-3}; sum of bases = 1.5 cmolc dm^{-3}; effective CEC = 5.5 cmolc dm^{-3}; Al saturation = 73%; CEC at pH 7 = 16.5 cmolc dm^{-3}; base saturation = 9.0%; organic matter = 11.7 g kg^{-1}; sand = 380 g kg^{-1}; silt = 420 g kg^{-1}; and clay = 500 g kg^{-1}) and irrigated with distilled water. Treatments were represented by two cultivars (IPR 91 and IPR 92) and five base saturation levels (9, 31, 53, 75, and 97%) in a 2 × 5 factorial arrangement. A completely randomized design with four replicates was adopted, using two plants per plot. After filling the pots, limestone was added, which reacted with the soil for 30 days. Subsequently, fertilization was carried out with the NPK 04-30-10 fertilizer equivalent of 200 kg ha^{-1}, in an adaptation of the method described by Görgen et al. (2016).

Stem diameter (SD), plant height (PH), number of branches (NB), number of leaves (NL), grain yield (GY), weight of 1000 mature grains with 13% moisture (thousand-grain weight, TGW), mature-grain moisture, and shoot fresh matter (SFM) were determined at harvest. Then, the samples (whole shoots) were weighed and air-dried (60 ºC) until reaching constant weight to determine the dry matter (SDM). Subsequently, the samples were ground and stored in a refrigerator (5 ºC) until the total-flavonoid and rutin contents and production were analyzed.

Preparation of extracts to determine total flavonoid and rutin contents

To obtain the extract, 10 mL of 80% methanol were added to 2 g of ground sample. The mixture was stirred for 6 h at room temperature, in the dark. Subsequently, the material was centrifuged at 4000 rpm for 10 min. After the process, the supernatant was removed, filtered through filter paper (11 cm in diameter, particle retention size of 4-12 μM), and stored, following the method adapted from Mendes, Martins, Fernandes and Marques (2005).

The sample was prepared by adding 5 mL of a 1% $AlCl_3$ aqueous solution and 7 mL of distilled water to 1 mL of the extract, vortexing for 20 s, then standing for 10 min in the dark at room temperature. Immediately after, readings were taken with the spectrophotometer (λ = 430 nm), using distilled water as the blank, in a method adapted from Pękal and Pyrzynska (2014).

The flavonoid content was measured in milligrams of rutin per gram of sample dry matter (mg g^{-1} DM), using the equation obtained by reading the rutin standard:

$$\hat{y} = 0.05 + 29.62x \ (r^2 = 0.99),$$

where "y" is the absorbance and "x" is the rutin concentration (mg mL^{-1}).

Flavonoid production was estimated by the equation:

$$\hat{y} \text{ (mg plant}^{-1}) = \text{Flavonoid content (mg g}^{-1} \text{ DM)} \times \text{Dry matter (g)},$$

where "ŷ" is flavonoid production per plant.

Determination of rutin content and production by liquid chromatography

The rutin content was determined by an adapted version of the method described by Zhai et al. (2018). The extracts were filtered through a sterile and hydrophobic Teflon membrane with 0.22-μm pores. Subsequently, they were injected into a high-performance liquid chromatography system with a diode array detector, in a VP-ODS C18 column (250 × 4.6 mm ID, 5 μm). Two mobile phases were used: acetonitrile (A) and water acidified with 0.5% phosphoric acid (B). The initial mobile phase was 80% B, then 70% B at 10 min, followed by a return to the initial composition at 25 min that was held until 35 min, when the runs were ended. The flow volume was 0.9 mL min^{-1}, column temperature set at 30 ºC, and sample injection volume of 1 μL. The runs were performed between 190 and 450 nm. The chromatogram peaks were integrated at the wavelength of 350 nm.

The rutin content was measured in milligrams per gram of sample DM (mg g^{-1} DM), based on the equation obtained from the reading of rutin standard:

$$\hat{y} = 793.71 - 20000000x \ (r^2 = 0.99),$$

where "ŷ" is absorbance and "x" is the rutin concentration (mg mL^{-1}).

Rutin production was estimated by the following equation:

$$\hat{y} \text{ (mg plant}^{-1}) = \text{Rutin content (mg g}^{-1} \text{ DM)} \times \text{Dry matter (g)},$$

where "ŷ" is rutin production per plant.

Statistical analysis

Statistical analysis was performed using the simple factorial arrangement model ($Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$), considering analysis of variance at 5% of significance by the F test. When significant, regression analysis was performed at 5% significance by the t test ($Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + ... + \beta_p X_i^p$) for base saturation, using R-Studio (2017).

Results and Discussion

Plant emergence occurred between seven and eight days after sowing (DAS). Inflorescence started at 20 DAS; flowers started to open at 26 DAS; and ripe fruits began to appear at 51 DAS. The number of branches (NB) and leaves (NL) per plant (4.6 ± 0.8 and 24.8 ± 8.5, respectively); root length (RL) (21.1 ± 5.9 cm); thousand-grain weight (TGW) (28.9 ± 36.3 g); total flavonoid (TFC) and rutin (TRC) contents (11.6 ± 3.3 and 11.7 ± 4 .0 mg g^{-1} DM, respectively); and total flavonoid (TFP) and rutin (RP) production (39.1 ± 16.8 and 38.8 ± 16.5 mg $plant^{-1}$, respectively) did not differ according to base saturation.

The number of branches of buckwheat in this study was higher than the 3.3-4.1 found in plants grown in China (Fang et al., 2018). The number of leaves, in turn, varied less compared with results (NL = 0-140) described by Aubert, Konrádová, Kebbas, Barris and Quinet (2020), who studied buckwheat grown in Amsterdam. These researchers counted NL every 10 days and summed the values at the end of the cycle. For this reason, the average may have reached higher values and with greater variation. The number of leaves found in the present study (NL = 18-28) was higher than the 23.9 reported in buckwheat lines grown in India (Bisht, Bhatt, & Singh, 2018a).

The total flavonoid content was lower than that found in buckwheat roots (20-50 mg g^{-1} DM) inoculated with Agrobacterium rhizogenes (Gabr, Sytar, Ghareeb, & Brestic, 2019). Among the flavonoids, roots have a rutin content of 9.3 mg g-1 DM and shoots 2.6-8.6 mg g^{-1} DM (Gabr et al., 2019), which are lower values than those observed in the present study. In European cultivars, total-flavonoid and rutin contents of 0.5-4.5 and 0.03-1.51 mg g^{-1} DM were detected, respectively (Kiprovski et al., 2015), both of which were lower than the present results (11.6 ± 3.3 and 11.7 ± 4.0 mg g^{-1}, respectively). Figure 1 illustrates the chemical profiles of cultivars IPR 91 and IPR 92, with their respective rutin spectra in the ultraviolet region.

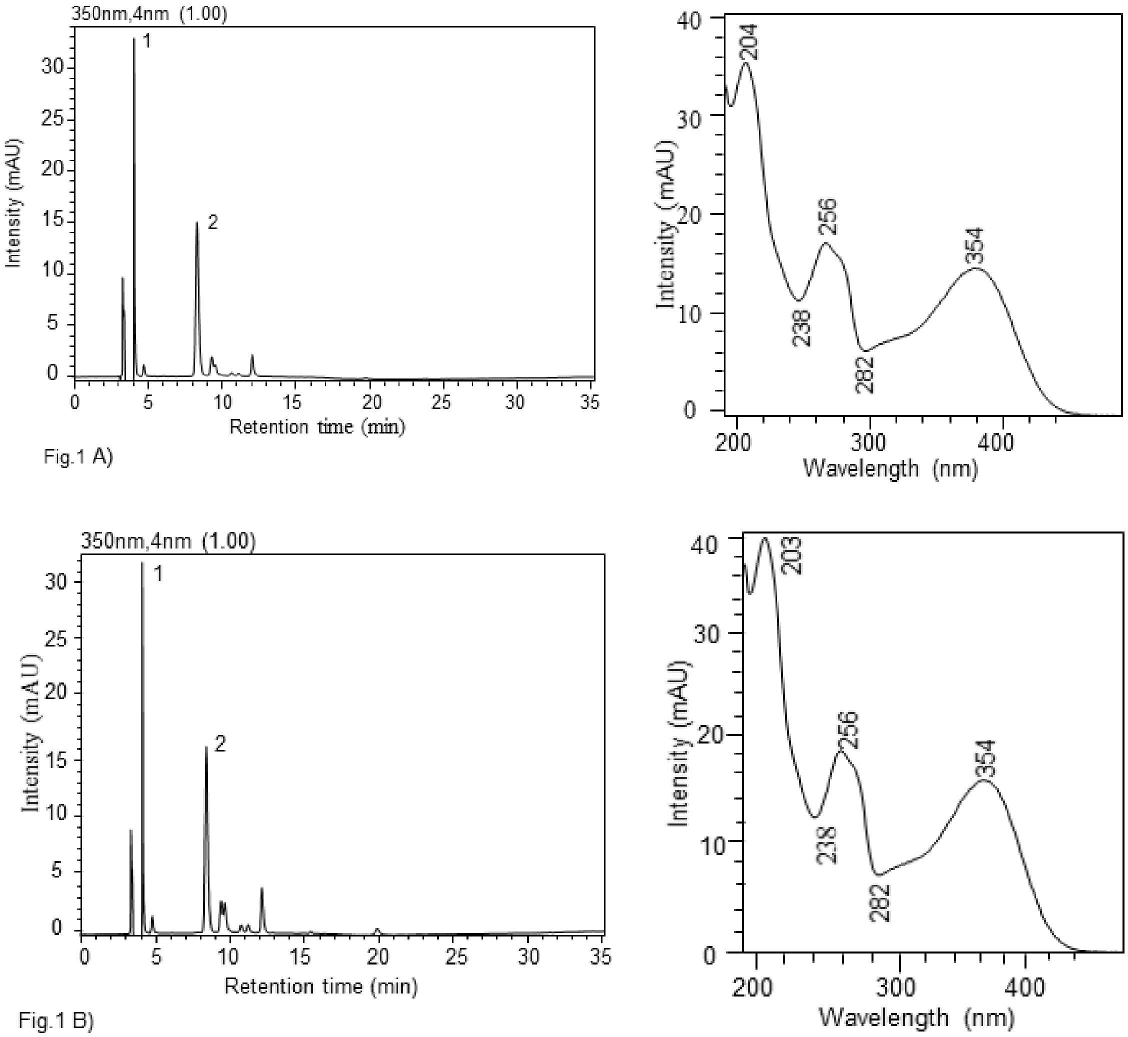

Fig.1 A)

Fig.1 B)

Figure 1. Chemical profiles, as obtained by high-performance liquid chromatography (at λ = 350 nm) of *Fagopyrum esculentum* (Moench) cultivars IPR 91 (A) and IPR92 (B) grown in a greenhouse in Minas Gerais, Brazil and the respective rutin spectra in the ultraviolet region. Peak 1: methanol solvent and peak 2: rutin.

The differences between the cultivars tested here and those studied in the literature, in addition to the standards used in the calibration curve in each analysis, contribute to the disparity of results. In our experiment, we used the rutin pattern, whereas in the afore-mentioned studies, researchers used the quercetin pattern. Total flavonoid content and production also vary according to the plant organ evaluated (Xiaohua et al., 2010). In the current study, the entire shoot was the object of investigation, aiming at optimal use of the plant. Biotic factors have a marked impact on flavonoid production (Siracusa, Gresta, Sperlinga, & Ruberto, 2017) and, therefore, different growing locations yield different results. The disparity of results can also be explained by the fact that HPLC and spectrophotometry systems are not all equal, with some being more sensitive than others.

Plant height (PH), shoot fresh (SFM) and dry (SDM) matter (Figure 2), root diameter (RD), and root fresh (RFW) and dry (RDM) matter (Figure 3) exhibited a negative quadratic response to increasing base saturation. There was a base saturation × cultivar interaction effect for stem diameter (SD) and grain yield per plant (GY) (Figure 4). Stem diameter exhibited negative-quadratic and positive-linear responses in cultivars IPR 91 and IPR 92, respectively, while GY showed no significant differences in IPR 91 and increased linearly in IPR 92, with increasing base saturation levels.

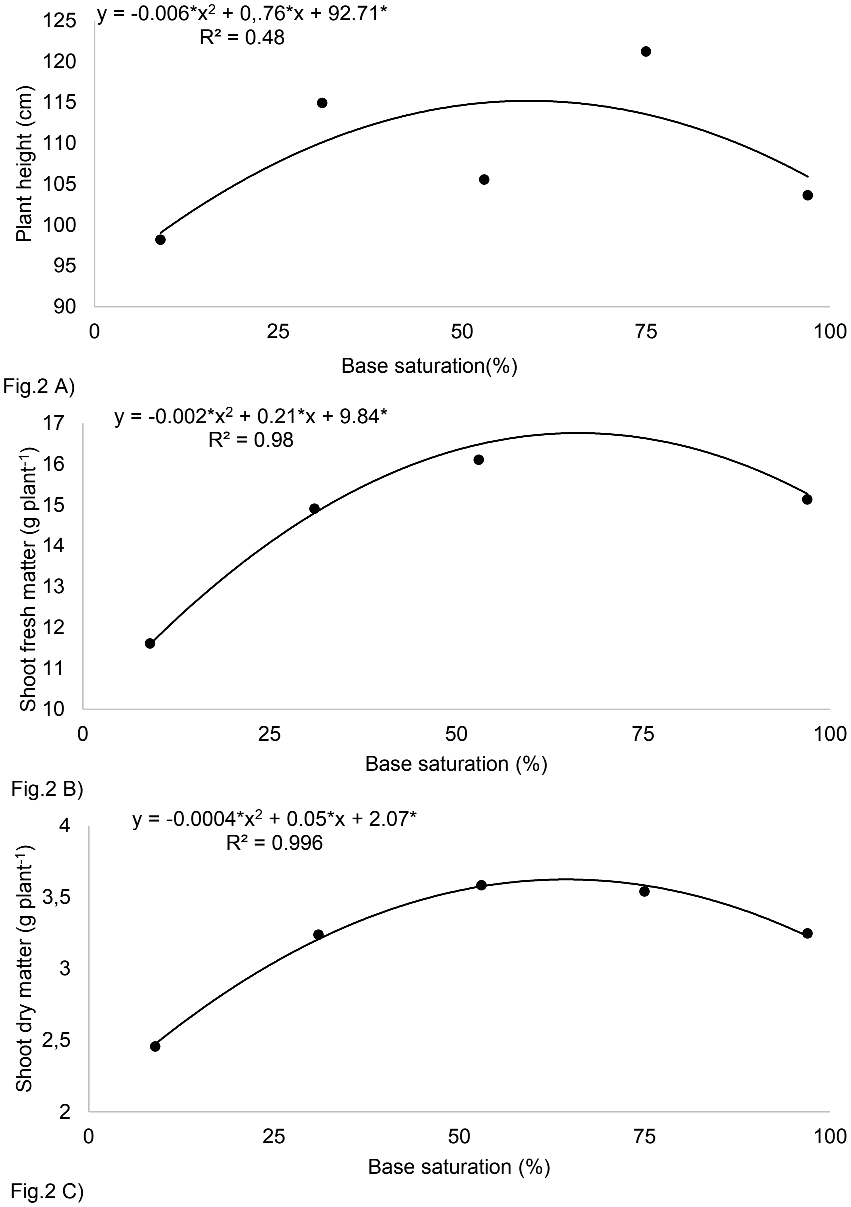

Figure 2. Plant height (A), shoot fresh matter (B), and shoot dry matter (C) of *Fagopyrum esculentum* (Moench) cultivars IPR 91 and IPR 92 as a function of base saturation in Minas Gerais, Brazil. *Coefficients significant by the t test at 5% significance.

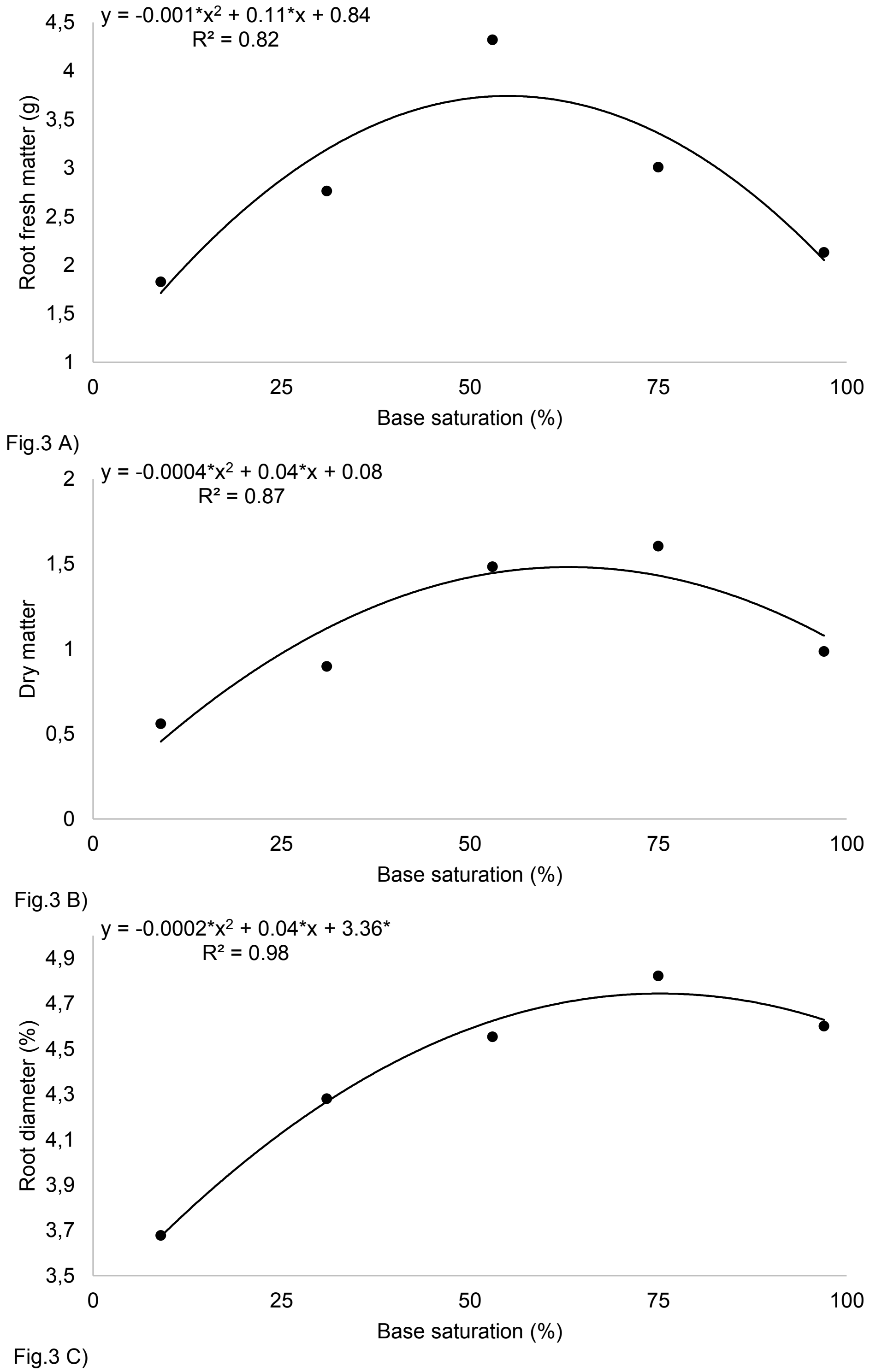

Figure 3. Root fresh matter (A), root dry matter (B), and root diameter (C) of *Fagopyrum esculentum* (Moench) cultivars IPR 91 and IPR 92 as a function of base saturation in Minas Gerais, Brazil. *Coefficients significant by the t test at 5% significance.

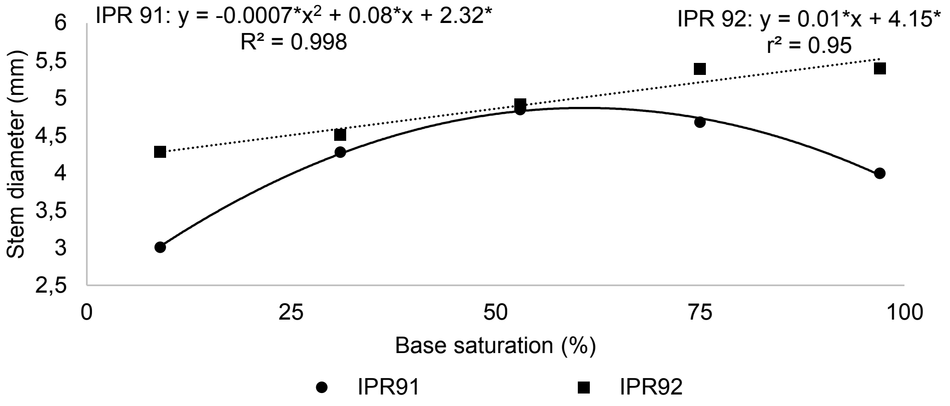

Fig.4 A)

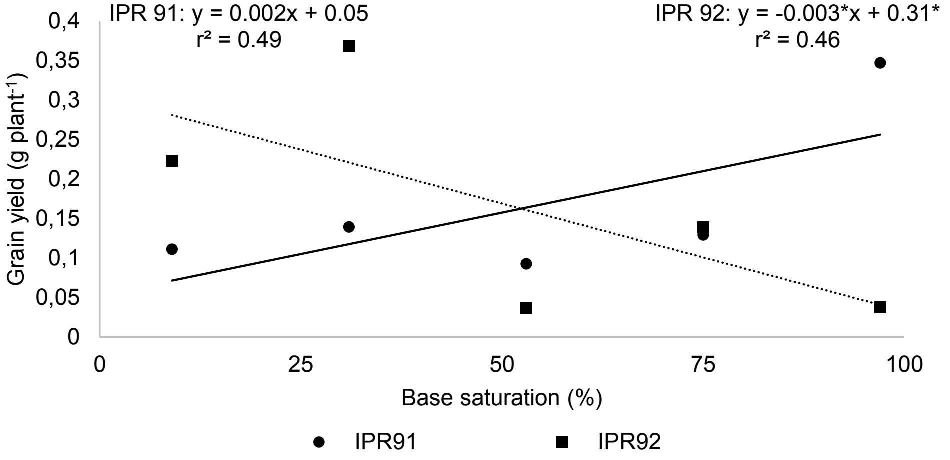

Fig.4 B)

Figure 4. Stem diameter (A) and grain yield at 13% moisture per plant (B) of *Fagopyrum esculentum* (Moench) cultivars IPR 91 and IPR 92 as a function of base saturation in Minas Gerais, Brazil. *Coefficients significant by the t test at 5% significance.

Base saturation influenced the roots, as buckwheat is an Al accumulator (Horbowicz et al., 2011; Xu et al., 2017). The roots of the species are not only important for the uptake and transport of elements to the seedling, but also effective in the plant's defense against Al (Horbowicz et al., 2011). When buckwheat is subjected to Al stress, they absorb less water, aquaporins are inhibited, and cell turgidity and elongation are also affected (Xu et al., 2017). For this reason, root-related variables tend to change according to potential and exchangeable acidity.

Most of the SFM and SDM means were lower than those described by Ribeiro et al. (2018), but higher than values reported in a study evaluating the sowing and seedling growth of buckwheat (1.62 and 0.22 g plant^{-1}, respectively (Podsiadło & Skorupa, 2017).

Ribeiro et al. (2018) evaluated buckwheat plants subjected to different types and rates of phosphates and reported PH values (40-130 cm) close to those found in this study. Stem diameter also was similar to the range described in *Fagopyrum tataricum* plants (4.1-5.8 mm) evaluated in two sowing dates in China (Xiang et al., 2019) and in buckwheat cultivars (3.4-4.5 mm) grown in Turkey (Unal, Izli, Izli, & Asik, 2017). The present results for PH and SD (Figures 2 and 3, respectively) also corroborate those reported by Fang et al. (2018) in a study on nitrogen fertilization and buckwheat planting density in China.

In this study, GY was lower than the 4.0-5.3 g plant^{-1} obtained by Jiang et al. (2018). Grain yield and TGW may have been lower because we adopted a grain moisture content of 13%, which is the ideal moisture level for the common-wheat crop (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2014). This reference is used because, to date, there is no predetermined moisture content for harvesting buckwheat. The TGW found in this study corroborates the results (24.7-28.1 g) described by Jiang et al. (2018), Siracusa et al. (2017), and Fang et al. (2018). In both cultivars, the highest SDM yield was achieved at base saturation levels close to 62%. With the exception of NL, NB, TGW, RC, RFW, SD, and GY, cultivar IPR 91 showed higher results than IPR 92 (Table 1).

Table 1
Characteristics of *Fagopyrum esculentum* cultivars grown in Minas Gerais, Brazil

Trait	Cultivar	
	IPR 91	IPR 92
Plant height (cm)	91.34b	126.08a
N of branches plant^{-1}	4.41a	4.78a
N of leaves plant^{-1}	23.47a	26.13a
Shoot fresh matter (g plant^{-1})	11.98b	17.93a
Shoot dry matter (g plant^{-1})	2.71b	3.72a
Root length (cm)	18.90b	23.33a
Root diameter (mm)	4.21b	4.57a
Root fresh matter (g)	2.33a	3.29a
Root dry matter (g)	0.85b	1.36a
Thousand-grain weight (g)	23.09a	34.65a
Flavonoid content (mg g^{-1})	10.26b	12.93a
Rutin content (mg g-^{1})	11.17a	12.16a
Flavonoid production (mg rutin plant^{-1}) by spectrophotometry	28.94b	49.23a
Rutin production (mg rutin plant^{-1}) by HPLC	31.52b	45.99a

HPLC: high-performance liquid chromatography. Means followed by the same letter in the rows do not differ statistically by the F test at 5% significance.

Base saturation was not an influential factor for most parameters. For grain and flavonoid production, the soil's natural base saturation (9.0%) should ideally be maintained. In addition to reducing production costs, this would provide an environment suitable for a higher GY in IPR 92. If the only objective of production is SDM, then the ideal scenario would be to keep base saturation close to 62.0%. Less saturation would be insufficient to meet the peak production of SDM, whereas higher levels would compromise production.

Shoot dry matter was positively correlated with PH, SD, RD, RFW, RDM, TFP, and RP (Tables 2 and 3). In future analyses, the determination of SDM will be sufficient to estimate these variables. Depending on the aim of production, it is possible to dispense with these assessments, maintaining only the measurement of SDM. For RP, SDM was more decisive than RC, as the latter did not correlate with RP. On the other hand, TFC had a strong significant correlation with TFP and RP, indicating that it is possible to determine these two variables from TFC. The total flavonoid content also had a significant positive correlation with PH. Thousand-grain weight only showed a significant correlation with grain yield, with higher GY values translating into greater TGW (Table 3).

Table 2
Correlation between traits in *Fagopyrum esculentum* cultivars IPR 91 and IPR 92 grown in Minas Gerais, Brazil, as a function of base saturation

	PH	SD	NB	NL	SFM	SDM	RL	RD	RFW	RDM
PH	1.00*	0.69*	0.31	0.16	0.88*	0.80*	0.48	0.54	0.56	0.54
SD		1.00*	0.04	0.07	0.80*	0.79*	0.29	0.85*	0.58	0.64*
NB			1.00*	0.84*	0.40	0.26	-0.24	0.31	-0.01	0.19
NL				1.00*	0.39	0.34	-0.24	0.30	0.04	0.20
SFM					1.00*	0.97*	0.39	0.78*	0.77*	0.79*
SDM						1.00*	0.44	0.78*	0.83*	0.78*
RL							1.00*	-0.04	0.56	0.18
RD								1.00*	0.59	0.72*
RFW									1.00*	0.87*
RDM										1.00*

*Significant by t test at 5% significance.

Table 3

Correlation between traits in *Fagopyrum esculentum* cultivars IPR 91 and IPR 92 grown in Minas Gerais, Brazil, as a function of base saturation

	GY	TGW	TFC	RC	TFP	RP
PH	0.23	0.53	0.65*	-0.01	0.85*	0.62
SD	-0.24	-0.06	0.48	-0.21	0.75*	0.54
NR	-0.11	0.16	0.15	0.49	0.33	0.52
NL	-0.26	0.06	0.29	0.72*	0.40	0.71*
SFM	0.02	0.39	0.62	0.08	0.96*	0.82*
SDM	0.02	0.39	0.54	0.04	0.93*	0.81*
RL	0.43	0.51	0.42	0.08	0.44	0.37
RD	-0.39	-0.06	0.21	-0.18	0.67*	0.54
RFW	0.05	0.37	0.48	0.01	0.78*	0.63*
RDM	-0.11	0.11	0.53	-0.02	0.79*	0.60
FY	1.00*	0.73*	-0.01	-0.27	-0.01	-0.18
TGW		1.00*	0.25	0.08	0.38	0.29
TFC			1.00*	0.48	0.80*	0.71*
RC				1.00*	0.25	0.61
PFT					1.00*	0.88*
RP						1.00*

*Significant by t test at 5% significance.

Conclusion

In the range of 9 to 97%, base saturation had no effect on buckwheat growing in terms of flavonoid production, but influenced shoot dry matter yield (62%) and grain yield in cultivar IPR 92.

Under the studied conditions, cultivar IPR 92 was higher-yielding than IPR 91.

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