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Effects of Drought on Yield and Nutraceutical Properties of Beans (*Phaseolus spp.*) Traditionally Cultivated in Veneto, Italy

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Abstract: Beans are often grown in regions with climates that are susceptible to drought during the cultivation period. Consequently, it is important to identify bean accessions tolerant to drought conditions and assess the effect of drought on seeds' nutraceutical properties. This study evaluated the effect of drought during different development stages (NES = never stressed; ALS = always stressed; SBF = stressed before flowering; SAF = stressed after flowering) on the yield and nutraceutical properties of six local bean varieties: Fasolo del Diavolo, Gialet, Posenati, Secle, D'oro, and Maron. Analysis of variance indicated that Gialet was not significantly affected by drought treatments, and Posenati under SBF and NES treatments had greater yields than under ALS and SAF treatments, whereas Secle under SBF produced 80% more seeds than under NES. Total phenols, antioxidant capacity, and calcium content were significantly different among the local varieties. Yield was significantly and positively correlated with seed calcium content and significantly and negatively correlated with protein, total phenols, and antioxidant capacity. The interaction between local varieties and treatment significantly affected seeds' Zn content. Gialet and Maron seeds' Zn contents were about 60 mg kg^{-1}, almost double the average of commercial varieties. In summary, this study paves the way to the identification of potential bean varieties resistant to drought. Further molecular studies will help support these findings.

Keywords: heirloom beans; drought; abiotic stress; local farming; nutraceutical properties; zinc

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1. Introduction

Legumes are essential to improve global food safety and security [1,2]. Common bean (*Phaseolus vulgaris*) is the most important directly consumed legume in the human diet [3] and the principal food legume for 250 million people in South and Central America and for 400 million people in Eastern Africa [4]. More than 40,000 varieties of beans are cultivated in all continents, except Antarctica, under different cropping systems and in a wide range of environments. Beans are considered one of the major crops with the highest levels of variation in growth habit, seed characteristics, maturity, and adaptation [5]. Beans also play an important role in achieving a more sustainable food production system [6–8] as they are considered an essential crop to help increase food production by 70% by 2050 in order to supply enough high-quality food to the world's growing population sustainably [9].

Legumes and beans are also gaining importance because of their health benefits preventing and helping to manage hypercholesterolemia, hypertension [10], obesity, diabetes, and coronary conditions [6]. Dry bean is known as a major source of protein, with a protein content from 20 to 30% of dry weight [11]. Thus, half a cup of beans (equivalent to 90 g) provides from 18 to 27 g of protein, which is from 32 to 48% of the recommended daily allowances (RDA) of a 70-kg adult (0.8 g of protein per kg of body weight; thus, 56 g) [12,13]. Beans also have considerable contents of phenols and flavonoids. Polyphenols usually are present in the seed coat, rarely in the cotyledons. [14,15]. Colored varieties tend to have a higher content of total phenols and greater antioxidant activities compared to non-pigmented ones [15,16]. Phenols can bring benefits such as reducing the risk of cancer, aging, apoptosis, and oxidative stress [17–19]. Antioxidants can be found in vegetable and fruits and prevent or delay some types of cell damage [20]. Dry bean is considered to be in both the vegetable group and the protein food group, as dry beans have similar protein profiles as foods in both groups and also provide other nutrients found in seafood, meats, and poultry, such as iron and zinc [21]. Indeed, legumes are a critical and affordable source of plant-based proteins, vitamins, and essential minerals such as calcium, magnesium, and zinc, contributing to the food security and nutrition of people around the world, especially subsistence smallholder farmers in developing countries [6]. Human and soil zinc deficiency can be correlated in Africa, Asia, Andean South America, and Mexico [22–24]. In these same regions, where beans are common sources of protein, beans can also be an excellent source of zinc for human diets [5,21]. Bean consumption in sufficient quantities is considered a strategic remedy for hidden hunger and healthy diets [25].

The domestication of common beans was unusual, occurring in two distinct geographic regions simultaneously, creating two gene pools, namely, the Middle American and the Andean [26]. The first introduction of common bean from Central/South America into Western Europe most likely took place in the sixteenth century [27,28]. In Italy, beans were introduced in 1515 [29] and created a long tradition that allowed the evolution of many local varieties adapted to microclimates in restricted areas [30]. In Veneto, the diffusion of common bean occurred quickly, and today, the cultivation of this pulse is of great economic relevance in the Belluno region [31]. However, although those local varieties have original morpho-agronomic and nutritional characteristics, with high organoleptic qualities, they have been gradually substituted by genetically uniform commercial varieties [30,32]. In order to conserve, preserve, and valorize this genetic material, the Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE) from the University of Padova is establishing a bean germplasm that currently contains 48 accessions, of which 27 are Venetian local varieties, with the preservation of functional and serependic opportunities afforded by plant species diversity for a more sustainable agriculture future.

Most of the world bean production areas are under risk of facing drought conditions during the cultivation cycles [33–36]. These areas are also under high and/or very high risk of human-induced desertification in upcoming years [37], indicating that the effects of drought conditions on bean production may be exacerbated. Drought can reduce bean yields by up to 75% [33,34] or, in more drastic conditions, can lead to total loss of production. At the same time, intensive irrigation systems can lead to an overuse of water and wasting the excess, which may lead to negative impacts on the environment. The long-term objective of the DAFNAE germplasm is to improve Italian and Venetian beans to be used in sustainable organic farming systems. Thus, it is important to better understand the plant development and agronomic performances of some of these local varieties under different environments and irrigation regimes to develop management strategies that can stimulate and support farmers to adopt these beans in their production systems. In addition, it is also important assess their nutraceutical properties in order to valorize this genetic material.

This study aimed to assess the effect of drought conditions during different growth stages of six local bean varieties traditionally cultivated in Veneto (Italy) on agronomic performance and nutraceutical characteristics. Fasolo del Diávolo, Gialet, Posenati, Secle, D'oro, and Maron were selected from the DAFNAE/UNIPD bean germplasm.

2. Materials and Methods

To ensure that the plant responses were primarily related to the drought stress, all the treatments applied were not restricted in terms of environmental factors (i.e., temperature, relative humidity, fertilization, operational practices), which were exactly the same for all cultivated plants, except the irrigation regime. Four different irrigation regimes were imposed on these local varieties: never stressed, always stressed (from sowing to harvesting), stressed before flowering, and stressed after flowering. The irrigation regimes were controlled by using a precipitation exclusion rain-out shelter and the irrigation was applied based on sensors in the pots measuring the volumetric water content every hour. The effect of these different irrigation regimes was assessed on the yield and seeds' antioxidant capacity, total phenols, phosphorus, zinc, calcium, magnesium, and protein content.

2.1. Plant Materials

The Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE) from the University of Padova (UNIPD), located in Veneto (Italy), is estabilishing a bean germplasm aiming to conserve beans traditionally cultivated in Italy and, more specifically, in Veneto. This germplasm is composed of 48 accessions and, for this study, we selected six accessions that were tradionally cultivated for centuries by small farmers in the mountainous region of Veneto and selected by them based on their agronomic performance, visual traits, and nutraceutical porperties. Fasolo del Diavolo (Devil's Bean) is a *Phaseolus coccineus*, also known as a runner bean. Gialet (yellowish) and Posenati (from Posina, Veneto) are *P. vulgaris* from the Middle American domestication center and Secle, D'oro (made of gold), and Maron (brown) are *P. vulgaris* from the Andean domestication center (unpublished data) (Figure 1).

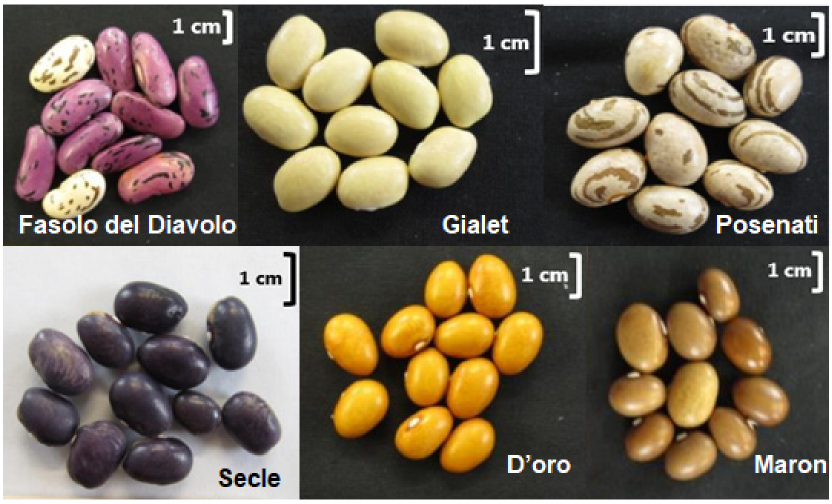

Figure 1. Seeds of landraces used in this study, showing the high diversity of visual traits and potential for commercial interests.

2.2. Experimental Design

A pot study was conducted in a rain-out shelter located in the state of Georgia, USA (33°46′53.8248″ N, 83°19′49.7028″ W) from March 2020 until August 2020. The rain-out shelter was completely covered on rainy days. On sunny and cloudy days, the sides of the rain-out shelter were removed, allowing air to flow through it. Each landrace was subjected to four treatments: never stressed (NES), always stressed (ALS), stressed before flowering (SBF), and stressed after flowering (SAF). From sowing until flowering, NES and SAF received a normal irrigation regime and ALS and SBF received a reduced irrigation regime. After flowering, NES and SBF received a normal irrigation regime, whereas ALS and SAF received a reduced irrigation regime. Pots were arranged in a randomized complete block design with four replications of each treatment. A Decagon Em50 data logger was placed in the middle of the shelter and connected to a VP-4 Humidity/Temperature/Barometer sensor (Meter Group Inc., Pullman, WA, USA)) to monitor and record air temperature and relative humidity every hour. The temperature inside the rain-out shelter ranged from 1.2 (2 April at 8 a.m.) to 38.5 °C (4 July at 3 p.m.), and the average temperature was 22.6 °C ± 8.3 (Figure 2a). Four 5TM Moisture/Temperature soil probes were placed at a 5 cm depth into different treatment pots to monitor hourly soil water content. Four reference pots without plants were placed randomly and weighed before and after each irrigation to monitor water evaporation. After the study was concluded, sensors were calibrated in the lab using the same soil with different water contents. Gravimetric water content was determined and converted to volumetric water content using the soil density. Unprocessed data were converted into volumetric water content (VWC, m^3 of water per m^3 of soil) using a calibration curve (R^2 = 0.9342), and the hourly average volume of water per pot of each treatment before and after flowering was calculated. The field capacity (FC) and the permanent wilting point (PWP) of this soil were 46 and 16% (VWC), respectively, or 1.62 and 0.56 L of water per pot. Plant available water was estimated as the difference between water content and permanent wilting point (Figure 2b).

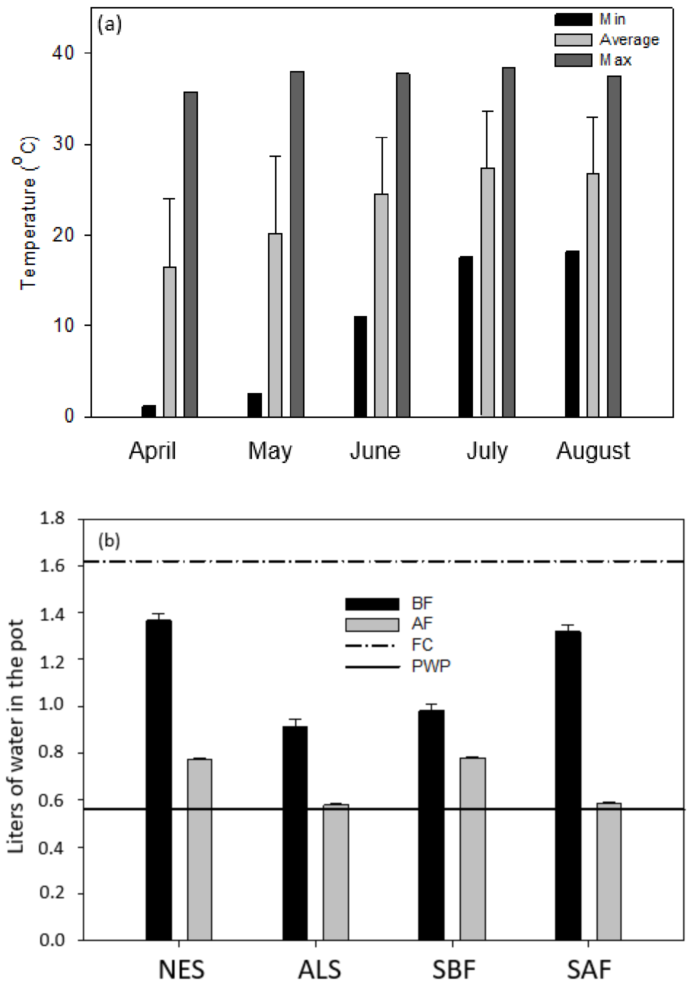

Figure 2. (**a**) Minimum, maximum, and average temperature inside the rain-out shelter from April 2020 until August 2020. (**b**) Hourly average of liters of water per pot in each treatment before flowering (black, BF) and after flowering (gray, AF). Field capacity (FC) is represented by the dash-dot line and permanent wilting point is represented by the solid line. Plant available water is the difference between the volume of water in the pot and the permanent wilting point. Treatments were (1) never stressed (NES), (2) always stressed (ALS), (3) stressed before flowering (SBF), and (4) stressed after flowering (SAF). The standard deviation is represented by error bars.

Each pot with a 20-cm diameter and 3.8 L volume was filled with 3 kg of certified organic soil compost (Soil Cube, Greenville, SC, USA) and two seeds were sowed on 30 March 2020, followed by 500 mL of water to saturate the soil. The soil used had a high organic matter content, 32% loss-on-ignition (LOI) [38], pH 6.8 in 0.01 M $CaCl_2$ (1:2) and 7.3 in distilled H_2O (1:2), bulk density of 0.85 g cm^{-3}, 22.8 mg N kg^{-1} of potentially mineralizable nitrogen by hot KCl methods, 57.5 mg kg^{-1} of plant available N, and CEC of 22.8 meq 100 g^{-1}. The C and N contents of the soil were 13.68% and 1.12%, respectively, which correspond to a C/N ratio of 12.2. Other nutrient contents were 4,845 mg Ca kg^{-1}, 1808 mg K kg^{-1}, 1270 mg Mg kg^{-1}, 632 P mg kg^{-1}, and 58 mg Zn kg^{-1}. Five days after planting (DAP), all plants emerged, and at three days after emergence, one plant was thinned from each pot, ensuring that all plants within each landrace were as homogenous as possible. All plants bloomed between 35 and 52 days after planting (DAP).

2.3. Plant Development and Yield

All five *P. vulgaris* have an indeterminate IIIb growth habit and *P. coccineus* IVa, according to the CIAT scale [39]. We used one cotton twine for each plant to support growth. One end was tied to the top of the rain-out shelter and the other end to the bottom of the plant. In the first weeks, we wrapped the stem around the twine, and the plants utilized the twine.

Every ten days, each plant development stage was monitored following the bean BBCH scale proposed by Feller et al. (1995) [40]. This scale has nine different growth stages, but for this study, the scale was adapted and summarized into four main growth stages:

i. Vegetative phase: from germination until first flower opens;
ii. Flowering: from the first flowers opening until the first pods are visible;
iii. Fresh pods: from the first green pod being visible until the first pod starts to dry and ripen;
iv. Dry pods: from when the pods and seeds ripen until the plant's senescence.

Pods were harvested when they appeared dry, and then seeds were left in a room with low relative humidity and temperature of about 20 °C, weighed, and the dry seed production per plant was determined.

2.4. Seeds' Nutraceutical Characterization

Air-dried seeds were ground to fine powder at room temperature, and approximately 0.2 g of each sample was added to a 50-mL Falcon tube and homogenized in 20 mL of methanol. Samples were filtered in Whatman 4 grade filter paper, and appropriate aliquots of extracts were assayed by Folin–Ciocalteau assay for total phenol content and by Ferric Reducing Antioxidant Power assay for total antioxidant activity. For all the nutraceutical properties analyzed, we used three technical replications for each biological replication.

The content of total phenols was measured on the basis of mg of gallic acid equivalent per kg (mg GAE kg^{-1} dw) of dry bean powder [41]. Bean extract was mixed with 20% sodium carbonate solution and Folin–Ciocalteau reagent [42]. After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm. The evaluation of total phenols was conducted utilizing a standard calibration curve (R^2 = 0.9995) of gallic acid solution with concentrations from 0 to 1600 µg mL^{-1} of gallic acid equivalent.

FRAP reagent was prepared fresh so that it contained 1 mM 2,4,6 tripyridyl-2-triazine and 2 mM ferric chloride in 0.25 M sodium acetate at pH 3.6 [43]. A 100-µL aliquot of the methanol extract prepared as above was added to 1900 µL of FRAP reagent and accurately mixed. After leaving the mixture at 20 °C for 4 min, the absorbance at 593 nm was determined. The evaluation of antioxidant capacity was conducted utilizing a standard calibration curve (R^2 = 0.9989) of ferrous ammonium with concentrations from 0 to 1200 µg mL^{-1} of Fe2+E (ferrous ion equivalent).

Approximately 0.2 g of dried ground bean powder was digested on two cycles of 200 °C for one hour and at 375 °C for two and a half hours, with 3 mL sulfuric acid being added at each cycle. Catalyst was added in the first cycle only. Total protein was calculated from total nitrogen measured from the Kjeldhal digestion [44], and total phosphorus was determined using the method described by Jirka et al. (1976) [45]. Calcium, zinc, and magnesium were measured in a Perkin Elmer AAnalyst 200 atomic absorption unit using standard curves obtained by diluting 1000 ppm stock solutions (Fischer Chemical) in 2% nitric acid.

2.5. Statistical Analysis

All data collected were digitized and processed on Microsoft Excel. A summary spreadsheet with the data of each variable of each experimental unit was made and input into JMP Pro 15 (JMP, version Pro 15, SAS Institute Inc., Cary, NC, USA, 1989–2019) that was used to perform the statistical analysis. The data were assessed for normality and tested for homogeneity in JMP Pro 15 and found to be normally distributed and homogenous. Analysis of variance (ANOVA) considering the main effects of treatment, variety, and their interaction was performed, and the Tukey test was used to separate means at $p < 0.05$. Principal component analysis (PCA) and the correlation between the variables were also performed. SigmaPlot 11.0 (Systat Software, San Jose, CA, USA) was used to prepare graphs.

3. Results

Analysis of variance and interactions in Table 1 show the incidence of interactions in some measured parameters. The structure of the presented Results and Discussion follows the order in which the parameters are presented in Table 1.

Table 1. Analysis of variance (ANOVA) table with the effects on different local varieties, of treatments, and of the interaction of variety and treatments on yield and nutraceutical properties of Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020, except Fasolo del Diavolo that did not produce seeds. The *p*-values are followed by the F-values in parenthesis.

Parameters	Varieties	Treatments	Varieties × Treatments
Yield (g per plant)	<0.001 (11.71)	<0.001 (14.57)	0.021 (2.56)
Total phenols (mg GAE kg^{-1} dw)	<0.001 (17.13)	0.065 (2.87)	0.798 (0.63)
Antioxidant capacity (mg Fe^{+2} kg^{-1} dw)	0.006 (4.57)	0.301 (1.22)	0.296 (1.21)
Protein content (%)	0.198 (1.64)	0.057 (3.10)	0.813 (0.60)
Total phosphorus content (mg kg^{-1})	0.196 (1.69)	0.698 (0.36)	0.053 (2.21)
Mg content (mg kg^{-1})	0.231 (1.49)	0.489 (0.73)	0.597 (0.85)
Ca content (mg kg^{-1})	<0.001 (8.72)	0.111 (2.32)	0.149 (1.43)
Zn content (mg kg^{-1})	<0.001 (30.43)	0.070 (2.83)	0.002 (4.33)
Degrees of freedom	4	3	12

Total degrees of freedom = 79.

3.1. Yield

The interaction between local varieties and treatment had a significant effect on yield (Table 1). Posenati NES and SBF and Secle SBF produced more than 10 g of seeds per plant. Posenati NES and SBF produced significantly more than Posenati SAF and ALS. Secle SBF yield was significantly greater than either Secle SAF or SBF. Gialet was the only local variety that did not have its yield significantly affected when comparing the treatments within it. D'oro SAF yield was significantly the lowest and Maron ALS did not produce seeds (Figure 3). Fasolo del Diavolo did not develop pods and therefore it did not produce seeds.

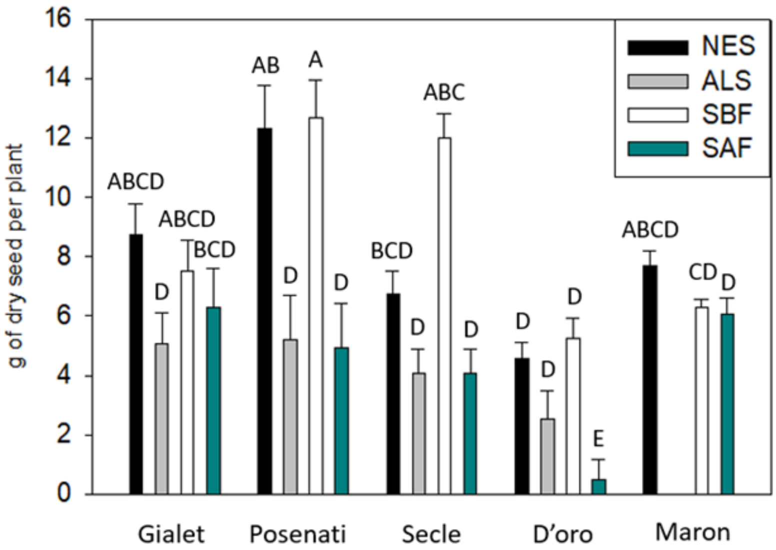

Figure 3. Effects of varieties and treatments interaction on yield (g of dry seeds per plant) of Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020. Different letters indicate a significant difference between compared groups. $\alpha = 0.05$. Treatments were (1) never stressed (NES), (2) always stressed (ALS), (3) stressed before flowering (SBF), and (4) stressed after flowering (SAF). Maron ALS did not produce seeds; thus, it is not represented in the graph. The standard deviation is represented by error bars (n = 4).

3.2. Nutraceutical Properties

Total phenols content was significantly affected by variety (Table 1). Maron and D'oro significantly had the greatest total phenols content, whereas Gialet significantly had the lowest (Table 2). The antioxidant capacity was also significantly affected by variety (Table 1). Maron had the greatest antioxidant capacity and Gialet the lowest, with a non-significant difference when compared to Secle (Table 2). Local varieties significantly affected the calcium content on the seeds (Table 1), with Gialet and Posenati having a significantly greater Ca content than Secle and D'oro (Table 2).

Table 2. Seeds' nutraceutical characteristics of five Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020.

Parameters	Gialet	Posenati	Secle	D'oro	Maron
Total phenols (mg GAE Kg^{-1} dw)	2455 (76) c	3368 (76) b	3326 (52) b	4288 (77) a	4128 (67) a
Antioxidant capacity (mg Fe^{+2} kg^{-1} dw)	2505 (36) c	3718 (85) b	3256 (88) bc	3736 (154) b	4545 (135) a
Protein content (% dw) n.s.	30.9 (4.4)	30.3 (2.4)	28.2 (3.4)	32.3 (2.8)	27.1 (3.5)
Total phosphorus (mg kg^{-1} dw) n.s.	1395 (36)	1386 (54)	1439 (81)	1422 (28)	1379 (55)
Mg content (mg kg^{-1} dw) n.s.	297 (13.16)	287.8 (14.7)	292.7 (12.4)	296.3 (6.5)	281.3 (11.1)
Ca content (mg kg^{-1} dw)	1280 (163) a	1118 (193) a	996 (194) b	945 (133) b	1081 (132) ab

Means followed by different letters in the same row indicate a significant difference between compared local varieties. n.s. = non-significant difference. $\alpha = 0.05$. The standard deviation is in parenthesis after the values (n = 4). Maron ALS did not produce seeds; thus, it is not represented in the table.

Zinc content was significantly affected by the interaction between variety and treatment (Table 1). Gialet SBF and SAF and Maron NES had Zn content greater than 60 mg kg^{-1}, whereas Posenati SBF and SAF and D'oro SAF had Zn content lower than 40 mg kg^{-1}. On average, Gialet and Maron zinc content was about 60 mg kg^{-1} (Table 3).

Table 3. Effects of varieties and treatment interaction on zinc content of Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020. Treatments were (1) never stressed (NES), (2) always stressed (ALS), (3) stressed before flowering (SBF), and (4) stressed after flowering (SAF).

Varieties	Treatment	Zn Content (mg kg^{-1})
Gialet	NES	58.4 (1.9) abcd
	ALS	56.8 (2.3) abcde
	SBF	61.8 (6.8) abc
	SAF	65.6 (6.7) ab
Posenati	NES	43.9 (2.7) efg
	ALS	47.4 (3.6) defg
	SBF	37.1 (5.2) g
	SAF	38.7 (2.4) fg
Secle	NES	47.9 (3.0) cdefg
	ALS	49.2 (1.8) bcdefg
	SBF	58.3 (8.6) abcd
	SAF	50.9 (4.8) bcdefg
D'oro	NES	49.4 (0.3) bcdefg
	ALS	56.2 (1.1) abcdef
	SBF	41.2 (1.6) fg
	SAF	37.5 (2.7) fg
Maron	NES	69.2 (0.3) a
	ALS	—
	SBF	58.3 (7.1) abcd
	SAF	51.4 (1.8) abcdefg

Means followed by different letters indicate a significant difference between compared groups. $\alpha = 0.05$. The standard deviation is in parenthesis after the values. Maron ALS did not produce seeds; thus, it is not represented in the table.

3.3. Principal Component Analysis and Correlations

Considering the relationship of yield with nutritional and nutraceutical parameters, a principal component analysis on covariance was performed. The total variation of C1 + C2 represented 98.4% of the total variability. Ca, Zn, and yield are on the C1 negative and C2 negative quarters, whereas total phenols and protein are on the C1 positive and C2 positive quarters. Antioxidant capacity is on the C1 positive and C2 negative quarters, and Mg and P are on the C1 negative and C2 positive quarters (Figure 4).

Yield correlation was significant ($p < 0.05$) and positive with calcium and siginificant and negative with total phenols, antioxidant capacity, and protein content. Total phenols and protein content were significant and positively correlated. Total phenols was significant and negatively correlated with zinc and calcium. Antioxidant capacity also had a significant and negative correlation with calcium and zinc (Table 4).

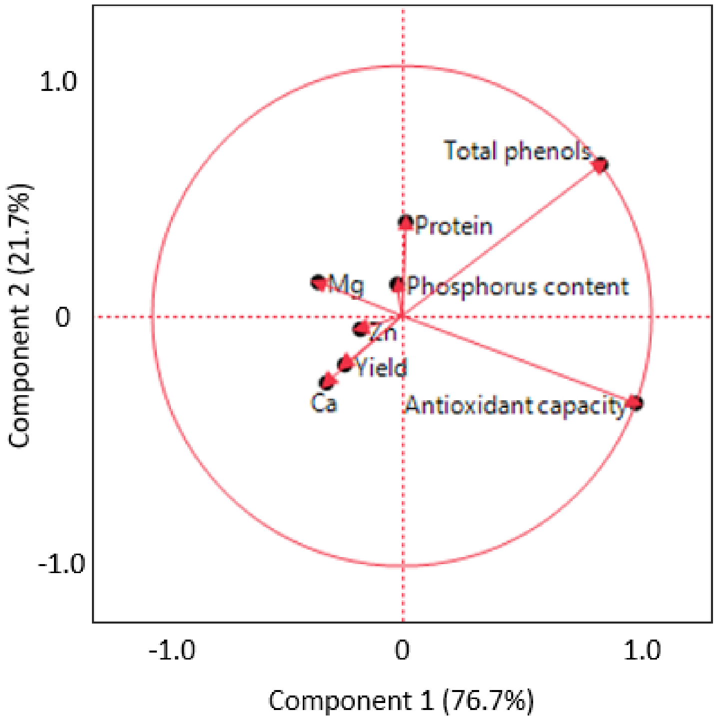

Figure 4. Principal component analysis (PCA) on covariance of nutraceutical properties of Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020.

Table 4. Correlations coefficients of the variables of Venetian bean (*P. vulgaris*) local varieties cultivated in a rain-out shelter between March and August 2020, estimated by the restricted maximum likelihood (REML) method.

	Yield	TP	AC	Protein	P	Zn	Mg	Ca
Yield	1							
TP	−0.5354 *	1						
AC	−0.3431 *	0.6675	1					
Protein	−0.2955 *	0.3749 *	−0.0078	1				
P	−0.1147	0.1566	0.0458	−0.1097	1			
Zn	0.0198	−0.2747 *	−0.2994 *	−0.1471	−0.0765	1		
Mg	0.0019	−0.1941	−0.2381	−0.0455	0.0997	−0.0504	1	
Ca	0.5062 *	−0.6477 *	−0.4166 *	−0.1714	−0.1023	0.2366	0.2335	1

TP = total phenols; AC = antioxidant capacity. * significant correlation ($p < 0.05$).

4. Discussion

This study assessed the effect of drought conditions at different growing stages on the yield and nutraceutical properties of six beans traditionally cultivated in Veneto, Italy. The local varieties had different responses to different irrigation regimes. Gialet was the only one that was not significantly affected by drought conditions and had a high zinc content. We also found that yield was significant and negatively correlated with protein content, total phenols, and antioxidant capacity. In this study, calcium was significant and positively correlated with yield.

Fasolo del Diavolo did not develop pods in this experiment, possibly due to two reasons: (i) *P. coccineus* has a high degree of allogamy, needing the presence of specific pollinators. We speculate that the rain-out shelter was not conducive to the presence of these pollinators or conditions within the rain-out shelter resulted in the reduced incidence of proper plant–pollinator contact [46]. (ii) *P. coccineus* tolerates temperatures up to 30 °C, and in this experiment, the temperature inside the rain-out shelter was commonly greater than 35 °C during the daytime. Thus, to assess the effects of drought conditions on this local variety, further research in an environment with a cooler temperature would be recommended. This also indicates that the cultivation of this local variety should be limited to the mountainous environment, where it originates, with milder temperatures throughout the growing period.

Polania et al.'s (2016) results showed that Middle American accessions tend to be more tolerant to drought conditions. The superior performance under drought stress of these accessions was associated with a better canopy biomass at mid-pod filling, which can be related to a deeper root system and effective use of water, an efficient remobilization of photosynthates, and grain filling [47]. Dipp et al.'s (2017) results confirmed that Andean accessions have a higher susceptibility to drought conditions [48]. In this study, Gialet and Posenati represented the Middle American gene pool. Drought conditions did not have significant effects on Gialet's yield, and Posenati's yield was not affected when it was under the SBF treatment. Maron and D'oro, two of the Andean local varieties, had higher susceptibility to drought stress; however, they had different responses. Maron ALS plants were more affected, whereas D'oro's SAF plants were more susceptible. Dipp et al. (2017) found that highly susceptible accessions can have yields reduced by about 70% and they considered that accessions with yield reduction lower than 40% are tolerant to drought conditions [48]. Smith et al. (2019) also found that drought conditions can significantly reduce yield by 70%. The yields of Gialet and Secle, for example, were reduced by 42 and 40%, respectively, under drought conditions during the whole cycle, indicating that these accessions can be tolerant to drought conditions [49].

Stressing the plant before flowering and applying a normal irrigation regime during the reproductive stage increased the yield of Posenati and D'oro and significantly increased Secle's yields by 80%. Imposing stress before flowering on beans' cultivation is a strategy recommended for Italian bean local varieties and commercial varieties [50–52]. Thus, irrigation management is a tool that can be used by farmers cultivating these local varieties, reducing the amount of water applied and increasing the seed production and the water use efficiency, without affecting the nutraceutical properties of the seeds.

Smith et al. (2019) also evaluated the effect of drought conditions on the macronutrient and micronutrient contents of bean seeds. Drought conditions did not affect the content of phosphorus, calcium, zinc, and magnesium [49], as was the case in our study. In this study, however, variety and treatments interaction had a significant effect on seed zinc content. Posenati and D'oro ALS seeds had significantly higher content than SBF. Nicoletto et al. (2019) assessed the effect of two different environments (30 and 351 m altitudes) on seed qualities of two Venetian local varieties, Gialet and Lingua di Fuoco, and their results showed that the environment significantly affected antioxidant capacity, total phenols, and calcium, magnesium, and phosphate content in seeds [53]. Although the growth environment affected these parameters, drought conditions did not have significant effects on seeds' nutraceutical characteristics. However, there was a significant difference in nutraceutical properties among the local varieties. D'oro and Maron had significantly greater total phenols content and Maron also had the greatest antioxidant capacity, whereas Gialet had significantly the lowest content of total phenols and antioxidant capacity among the local varieties. Most of the antioxidants and total phenols of beans are present on the seed coat and colored seeds are related to high contents of these compounds [14–16], confirming the results of this study, as Gialet seeds are yellowish and pale, whereas D'oro and Maron have strong golden and brownish colors, respectively. In this study, local varieties from the Andean domestication center tended to have, on average, greater antioxidant capacity and total phenols content and lower calcium content, when compared to Middle American local varieties. Nicoletto et al.'s (2019) results for antioxidant capacity and total phenols of Gialet seeds were lower than the ones obtained in this study [53]. However, these results are based on the weight of dry seeds and previous studies have shown that soaking and cooking beans can reduce by 90% the total phenol content and antioxidant capacity [54–56].

Blair et al. (2009) considered a Middle American accession with 42.8 mg kg^{-1} Zn content on the seeds as a low content and an Andean accession with 66.7 mg kg^{-1} as high

Zn content [57]. Amongi et al. (2018) assessed the Zn content of 304 accessions of a common bean germplasm from East Africa. The Zn content on African accessions ranged from 23.9 to 47 mg kg^{-1}, with an average of 32 mg kg^{-1} [58]. This study obtained a similar range to Blair's, from 42.2 (Posenati) to 59.3 and 59.9 mg Zn kg^{-1} (Maron and Gialet, respectively). These local varieties had greater Zn concentrations' than modern varieties assessed by Celmeli et al. (2018) that ranged from 25 to 35 mg Zn kg^{-1} [59]. The average daily recommended amount of Zn consumption for an adult male is 11 mg [60], which means that if one adult male consumes the equivalent of 100 g of dry beans from a commercial variety containing 32 mg Zn kg^{-1}, it will provide about 29% of the recommended daily consumption of Zn. If the same man eats the same amount of dry beans from a Zn-rich local variety—such as Gialet and Maron, for example—it will provide about 55% of the recommended daily consumption of Zn in one meal. Paredes et al. (2010) assessed the macronutrient and micronutrient content of 52 Chilean bean accessions that had Mg content ranging from 1300 to 1800 mg kg^{-1}, about five times greater than the ones obtained in this study. In contrast, Smith et al. (2019) measured Mg magnesium contents closer to the ones from this study, about 500 mg kg^{-1}. In terms of Ca content, this study's results are closer to those of Paredes et al. (2010), which ranged from 1000 to 2100 mg Ca kg^{-1}, whereas Smith et al. (2019) found Ca contents around 500 mg kg^{-1}. Paredes et al. (2010) used an atomic absorption unit to determine Ca and Mg, as was used in this study, and Smith et al. (2019) used inductively coupled plasma (ICP) [49,61].

Table 4 indicates that the yield correlation with protein was negative (−0.2955) and significant ($p < 0.005$), in agreement with the results of Bulyaba et al. (2020) and Kazai et al. (2019) [62,63]. Davis et al. (2004) proposed a trade-off theory for the interaction between yield and nutrient content [64]. Thus, we hypothesize that, in this study, the trade-off of reduced protein content may be explained by the dilution effect since the increase in yield may result in a greater carbohydrates content and reduced nitrogen content within the endosperm. Total phenols and antioxidant capacity were also negatively correlated with yield for beans. Kruger et al. (2013) found that both were also negatively related to the yield of 23 accessions of black currants [65]. It is likely that greater yields increased the endosperm vs. coat ratio, and since most of the antioxidants and phenols are in the seed coat [14–16], their contents were decreased as yield increased, leading to the negative correlation found in this study. On the contrary, yield had a positive and significant correlation with calcium content (0.5062, $p < 0.05$). Ribeiro et al. (2013) found out that yield was positively correlated with Ca content (0.1566) [66]. However, in another study, Ribeiro et al. found a non-significant negative correlation between yield and calcium content [67]. Thus, we hypothesize that the correlation between yield and calcium content in the seeds may be explained by another factor besides the dilution effect, for example, a high Ca content in the soil, soil pH, different varietal requirements and Ca uptake, seed nutritional characteristics, and response to drought conditions. Domingues et al. (2016) found that a high bioavailability of Ca is correlated with higher yields and higher Ca content in the seeds. Since the soil used in this study had a high Ca content, we hypothesized that these local varieties were able to take advantage of the higher calcium content in the soil [68].

5. Conclusions

Exposure to drought conditions until flowering did not significantly reduce yields for any of the local varieties and instead increased Secle's yield by 80% when compared to the never stressed treatment. Thus, reducing irrigation before flowering is a tool that can be used by farmers cultivating these varieties to increase yield and their water use efficiency. Gialet was the only variety that did not have yields significantly affected by drought conditions for any of the treatments: before flowering, after flowering, and throughout the cultivation cycle. Maron did not produce seeds when exposed to drought conditions throughout the cultivation cycle. Drought conditions during different growing stages did not affect seeds' nutraceutical properties, except for zinc which was significantly affected by the interaction between variety and treatment. Gialet and Maron had a high zinc content when compared to commercial varieties (60 mg kg^{-1}). Gialet, a traditional variety from Veneto, has good potential of success when cultivated in areas under risk of facing drought conditions and is a good candidate for addressing the zinc deficiency faced by a significant proportion of the world's population. While these local varieties have some cultivation limitations for large commercial growers (i.e., indeterminate growth, manual harvesting, and practices to increase their efficiency and productivity are still little known), this study provides useful information to small and medium farmers that will help them to increase the water use efficiency and the nutritional values of these beans, which can be grown in other drought regions of the world. It is also possible that these results will help and promote the commercial value of these varieties and raise the interest of farmers to start producing them on larger scales. However, more studies in different environments under drought conditions are still needed in order to provide more information to bean growers.

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