DOI: 10.5433/1679-0359.22v43n5p2323

Sample sizing for common bean traits in irrigated and non-irrigated conditions

Dimensionamento amostral de caracteres de feijão em condição hídrica irrigada e não irrigada

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Highlights

The sample size differs in irrigated and non-irrigated conditions.
The sample size for 18 traits varies according to the common bean cultivar.
Sample sizing increases accuracy in data collection.

Abstract

The use of statistical methods to evaluate plant growth and production is crucial for the technological advancement of common bean. The aim of this study was to determine the sample size necessary to estimate the mean of traits evaluated in different cultivars and irrigation conditions. Data were collected fortnightly from two 3 × 2 factorial experiments (three cultivars: Triunfo, Garapiá, and FC104; two irrigation regimes: irrigated and non-irrigated). Eighteen traits were evaluated (height, stem diameter, number of nodes, root length, shoot and root fresh and dry weights, leaf temperature, leaf area, number of nodules, nodule fresh and dry weights, number of pods, pod length, grains per pod, grains per plant, and grain dry weight). The sample size was determined using the bootstrapping resampling method from 2,000 resamplings, and was defined as the number of plants at which the 95% confidence interval was 10% to 40% of the estimate of the mean. As a result, the sample size differs between traits and between the cultivars and irrigation conditions

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Received: June 07, 2021 - Approved: Nov. 07, 2022

tested. In the 95% confidence interval with a standard error of 40% of the estimate of the mean, to evaluate all analyzed traits, 44 plants are needed for the shoot traits, 132 for the root traits, and 12 for the yield traits. To analyze the 18 studie0d traits, 132 plants are required.
Key words: Experimental precision. Phaseolus vulgaris. Resampling.

Resumo

O uso de métodos estatísticos para avaliar o crescimento e produção das plantas é crucial para o avanço tecnológico do feijoeiro. A pesquisa teve por objetivo dimensionar a amostra para a estimação da média de caracteres avaliados em diferentes cultivares e condições hídricas. Os dados foram coletados quinzenalmente a partir de dois experimentos fatoriais 3 x 2 (3 cultivares: Triunfo, Garapiá e FC104; 2 regimes hídricos: irrigado, não irrigado). Foram coletados dezoito caracteres (estatura, diâmetro da haste, número de nós, comprimento da raiz, massa fresca e seca da parte aérea e raízes, temperatura foliar, área foliar, número de nódulos, massa fresca e seca dos nódulos, número de vagens, comprimento das vagens, grãos por vagem, grãos por planta e massa seca dos grãos). O tamanho da amostra foi determinado através do método de reamostragem boodstrap a partir de de 2.000 reamostragens, e foi definido pelo número de plantas a partir das quais o intervalo de confiança de 95% foi de 10% a 40% da estimativa média. Como resultado, o tamanho da amostra é diferente entre os caracteres e entre as cultivares e condições hídricas utilizadas. No intervalo de confiança de 95% com erro padrão de 40% da estimativa da média, para avaliar todos os caracteres analisados são necessárias 44 plantas dos caracteres de parte aérea, 132 plantas para os caracteres de raiz e 12 plantas nos caracteres produtivos. Para analisar os dezoito caracteres estudados são necessárias 132 plantas.
Palavras-chave: Phaseolus vulgaris. Precisão experimental. Reamostragem.

Introduction

The common bean (*Phaseolus vulgaris* L.) is an important component of food and nutrition security. It is produced and consumed in all regions, constituting one of the main sources of protein for the population (Los et al., 2018). Despite the need for increased production to meet world demand, the average Brazilian common bean yield in 2019/2020 was 1,104 kg.ha^{-1} (Companhia Nacional de Abastecimento [CONAB], 2020), a value lower than the potential 3,000 kg.ha^{-1} achievable in irrigated crops (Justino et al., 2019). One of the main factors that reduce common bean yield is the stress caused by soil water deficit (Schwerz et al., 2017), which results in decreased shoot and root cell expansion. Depending on the intensity, water stress can also negatively affect the plant's productivity and even senescence (Taiz et al., 2017).

In view of the search for mitigating the production deficit and for the sustainability of agroecosystems, scientific research must continue to be developed. The scarcity of time and human and financial resources limit the evaluation of the entire experimental unit; thus, sampling is an option to portray the population in a representative manner (Storck et al., 2011). Defining the sample size or the number of plants to determine the mean of a response variable, considering the irrigation management, is essential for measuring vegetative and reproductive traits with experimental precision, thus avoiding underestimation or overestimation.

In the sample size determined by resampling, the elements that will be part of the sample can be selected more than once through sampling with replacement (Ferreira, 2015). The bootstrapping resampling method has been used for defining the sample size and is independent of the probability distribution of the data (Ferreira, 2009). This procedure has already been used to determine the sample size for traits evaluated in flax (Cargnelutti et al., 2018a), jack bean (Cargnelutti et al., 2018b), dwarf pigeon pea (Cargnelutti et al., 2018c), and cassava (Schoffel et al., 2020).

Cargnelutti et al. (2008) established the sample size for reproductive traits in common bean and found the value of 10 plants for the height of insertion of the first and last pods, number of pods per plant, and grains per plant and per pod. However, the literature lacks information on sample sizing for the common bean crop in irrigated and non-irrigated conditions and for vegetative traits. Therefore, this study was undertaken to determine the sample size in number of plants necessary to estimate the mean of traits measured in cultivars Triunfo, Garapiá and FC104 under irrigated and non-irrigated conditions.

Material and Methods

Two experiments were carried out in a 150-m² screened shelter covered with 200-µm low-density polyethylene and with side walls lined with anti-aphid screen, located in the Department of Phytotechnology at the Federal University of Santa Maria (UFSM), Santa Maria - RS, Brazil (29°43′ S, 53°43′ W, 95 m asl). The first experiment took place from August to December 2019 and the second from January to April 2020, in a 3 × 2 factorial arrangement consisting of three common bean cultivars (Triunfo, Garapiá, and FC104) and two irrigation regimes (irrigated and non-irrigated) in a completely randomized design. Irrigation was conducted in a factorial arrangement individually for each pot, with each pot corresponding to a replicate. Table 1 shows the number of plants evaluated by combination of cultivar vs. irrigation condition. The number was lower for the nodulation traits, as they were non-existent at the beginning of the crop cycle; and also for the yield components, as the plants were collected fortnightly throughout the cycle, leaving fewer plants for harvest. In addition, sick plants or plants with an interrupted apical apex were discarded. Cultivars Garapiá and FC104 belong to the carioca grain group, and Triunfo to the black grain group. Each experimental unit consisted of an 8-L pot with one plant that was filled with typic alitic Argisol (Hapludalf) (Santos et al., 2018).

Table 1
Number of plants used for each trait analyzed in cultivars Triunfo, Garapiá, and FC104 in irrigated and non-irrigated regimes.

	Triunfo		Garapiá		FC 104	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated
Height	81	77	83	82	67	65
Number of nodes	81	77	83	82	67	65
Stem diameter	63	60	65	64	52	50
Root length	63	60	65	64	52	50
Shoot fresh weight	63	60	65	64	52	50
Shoot dry weight	63	60	54	64	52	50
Root fresh weight	57	60	65	62	52	50
Root dry weight	63	60	65	64	52	50
Number of nodules	43	35	58	54	33	25
Nodule fresh weight	36	27	51	45	27	16
Nodule dry weight	38	28	52	47	28	17
Leaf area	42	41	49	43	40	39
Leaf temperature	145	139	139	135	190	162
Number of pods	23	20	26	24	21	20
Pod length	23	20	26	24	21	20
Grains per pod	23	20	26	24	21	20
Grains per plant	23	20	26	24	21	20
Grain dry weight	23	20	26	24	21	20

The irrigation conditions were imposed using the fraction of transpirable soil water (FATS) method at R5 (Fernández et al., 1986) for all cultivars in the first experiment and for Triunfo and Garapiá in experiment 2, whereas FC104 in the last experiment was subjected to water deficit at the V4 vegetative stage (Fernández et al., 1986). Plants under water deficit were not irrigated until they showed 10% of the transpiration of the irrigated plants, which had their amount of transpired water replenished daily, according to the method proposed by Sinclair & Ludlow (1986).

Vegetative traits data were collected fortnightly from emergence to harvest in both experiments. All data collected throughout the plant's growth and development in the two experiments were pooled and divided into combinations of cultivar vs. irrigation condition. Sowing time was not considered a factor.

The following data were determined: height of the main stem (H, cm), measured from the ground to the last node, with a millimeter ruler; number of nodes (NN), measured from the node of unifoliate leaves to the last node with a fully expanded trifoliate;

diameter of the main stem (SD, cm), measured between the cotyledonary node and the node of unifoliate leaves, with a caliper; root length (RL, cm), measured with a millimeter ruler; fresh and dry weights of shoots (SFW and SDW) and roots (RFW and RDW) (g), by weighing immediately after collection and drying at 65 ºC until constant weight and weighing again; number of nodules (NDL) with a diameter greater than 2 mm and their respective fresh and dry weights (NFW and NDW), which were weighed immediately after counting and dried at 65 ºC until constant weight and weighed again; leaf area (LA, cm plant^{-1}) determined by the equation LA = 1.092C1.945 (Pohlmann et al., 2021) during stages V4 to R8 (Fernández et al., 1986); leaf temperature (LT, ºC), measured daily at 15h00 during the water deficit in the central leaflet of the trefoil located in the upper third, using an infrared thermometer. The following data referring to yield components were collected at the end of the crop cycle at R9: number of pods (NP); number of grains per plant (GPl) and per pod (GPo), by counting; pod length (PL), measured as the distance between the ends of the pod with a millimeter ruler; and dry weight of grains at 13% moisture (GDW), which were weighed after drying in the sun.

For each measured trait, the 25th and 75th percentiles, variance, standard deviation (SD), and coefficient of variation (CV) were calculated using IBM SPSS software version 22.0 (International Business Machines, Statistical Package for the Social Sciences [IBM SPSS], 2021). To calculate the sample size, an iterative process was carried out with 2,000 resamplings with replacement, using different sample sizes (n), starting with 2 and adding 1 in each iteration up to a maximum size of 1,000 readings, thus generating the 2,000 means for each of the 999 sample sizes used (Ferreira, 2009). The plant sample size was defined as that at which the 95% confidence interval (AIC95%) was equal to 10, 15, 20, 25, 30, 35, and 40% error of the estimate of the mean (Ferreira, 2009). Analyses were performed using R software (R Core Team [R], 2020).

Results and Discussion

The variance, SD, and CV values were higher in irrigated than non-irrigated plants for most of the evaluated traits, except SD, RDW, LT, and GPo in Triunfo; SD, RFW, RDW, and LT in Garapiá; and NN and LT in FC 104 (Table 2). Water deficit initially affects cell expansion by reducing cell turgor and later induces stomatal closure and a reduction of photosynthetic rate (Taiz et al., 2017). In this way, non-irrigated plants suffer growth and development limitations, which may have resulted in a smaller amplitude of the data and more uniform plants. Another factor that may have influenced the result was the non-uniform irrigation during the period of water deficit, as the water lost through transpiration in the irrigated plants was replaced daily and this value varied in each plant, causing greater variability in the irrigated plants.

Table 2
Variability of the traits of height (H), number of nodes (NN), stem diameter (SD), root length (RL), fresh and dry weight of shoots (SFW and SDW) and roots (RFW and RDW), leaf area (LA), number of nodules (NDL), nodule fresh and dry weights (NFW and NDW), leaf temperature (LT), number of pods (NP), pod length (PL), grains pod^{-1} (GPo), grains plant^{-1} (GPl), and grain dry weight (GDW) of common bean cvs. Triunfo, Garapiá, and FC104 under irrigated and non-irrigated conditions.

	P25%	P75%	Variance	SD	CV	P25%	P75%	Variance	SD	CV
	Triunfo Irrigated					Triunfo Non-irrigated				
H	61.2	130.9	2182.8	46.7	48.5	57.0	103.1	1380.3	37.2	48.0
NN	10.0	13.0	13.8	3.7	33.9	9.0	12.0	11.9	3.5	34.0
SD	0.5	0.8	0.0	0.2	29.7	0.5	0.8	0.0	0.2	31.7
RL	43.0	65.0	454.1	21.3	40.8	43.0	62.5	416.2	20.4	40.9
SFW	30.6	75.0	1204.4	34.7	65.3	30.9	59.0	758.3	27.5	59.9
SDW	6.1	29.3	159.0	12.6	65.4	5.9	22.8	89.0	9.4	63.3
LA	750.9	1734.0	364583.4	603.8	46.8	773.1	1092.1	78574.0	280.3	30.2
RFW	19.0	46.7	622.5	25.0	72.6	19.8	48.8	461.2	21.5	67.5
RDW	3.4	7.9	15.9	4.0	67.4	3.1	8.0	22.4	4.7	79.9
NDL	28.0	205.0	24130.4	155.3	108.2	12.0	154.0	6486.3	80.5	83.1
NFW	0.5	1.9	2.6	1.6	103.5	0.0	2.3	1.2	1.1	78.1
NDW	0.1	0.4	0.1	0.3	115.1	0.1	0.3	0.0	0.2	78.6
LT	22.3	28.0	15.8	4.0	15.7	23.5	30.2	28.2	5.3	19.8
NP	11.0	14.0	8.5	2.9	22.9	9.0	12.8	5.6	2.4	22.0
PL	9.1	10.4	0.8	0.9	8.9	8.8	9.7	0.5	0.7	7.5
GPo	4.2	5.4	0.6	0.8	15.8	3.8	4.8	0.6	0.8	19.3
GPl	49.0	74.0	317.0	17.8	29.1	34.3	49.8	104.2	10.2	23.6
GDW	11.6	19.0	20.8	4.6	30.3	8.1	12.9	7.8	2.8	25.8
	Garapiá Irrigated					Garapiá Non-irrigated				
H	38.0	102.0	1417.9	37.7	53.4	32.0	88.4	1168.4	34.2	58.3
NN	9.0	13.0	11.5	3.4	32.0	10.0	12.0	9.8	3.1	30.9
SD	0.6	0.7	0.0	0.2	25.0	0.5	0.7	0.0	0.2	26.3
RL	51.9	70.8	421.6	20.5	35.5	47.5	67.5	400.4	20.0	36.0
SFW	41.8	80.1	1298.6	36.0	58.6	42.0	74.3	817.7	28.6	51.1
SDW	8.1	28.7	151.6	12.3	67.5	8.9	23.9	77.1	8.8	56.0
LA	877.6	1927.1	581402.9	762.5	52.9	895.6	1153.9	275522.7	524.9	51.1
RFW	22.4	60.0	708.6	26.6	64.2	20.0	70.0	979.1	31.3	69.0
RDW	3.8	8.8	21.3	4.6	67.6	3.9	9.4	23.7	4.9	68.9
NDL	30.8	202.3	24362.0	156.1	103.1	55.5	260.0	18735.2	136.9	78.9
NFW	0.8	3.3	4.0	2.0	91.8	0.2	3.2	2.5	1.6	62.7
NDW	0.1	0.4	0.1	0.3	91.7	0.2	0.4	0.1	0.2	71.5
LT	22.3	28.3	17.5	4.2	16.6	22.3	29.9	32.4	5.7	21.4

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NP	12.8	19.0	16.1	4.0	25.4	9.0	11.8	7.0	2.6	25.3
PL	8.5	10.1	0.8	0.9	9.4	8.4	9.5	0.5	0.7	8.0
GPo	3.8	5.3	0.7	0.9	18.8	3.8	5.0	0.6	0.8	18.5
GPl	54.0	86.3	267.4	16.4	23.3	39.3	51.8	99.4	10.0	22.6
GDW	12.5	20.8	15.8	4.0	24.0	9.0	11.7	6.5	2.6	24.8
	FC104 Irrigated					FC104 Non-irrigated				
H	54.5	126.1	2119.0	46.0	51.2	59.5	122.0	2012.7	44.9	50.3
NN	10.0	14.0	17.5	4.2	34.9	10.5	15.0	18.5	4.3	35.6
SD	0.5	0.6	0.0	0.1	24.1	0.4	0.5	0.0	0.1	20.6
RL	39.3	58.6	199.5	14.1	30.1	38.0	55.0	182.5	13.5	30.0
SFW	27.3	58.8	613.1	24.8	58.2	21.0	43.2	413.1	20.3	61.2
SDW	6.6	20.5	77.9	8.8	61.4	5.1	15.0	35.8	6.0	57.4
LA	744.2	1736.3	714739.0	845.4	62.9	655.0	1115.9	155253.5	394.0	45.9
RFW	14.8	44.0	447.4	21.2	66.9	10.0	25.2	340.3	18.5	85.5
RDW	4.2	8.9	26.5	5.2	71.4	2.5	7.1	10.4	3.2	72.7
NDL	14.0	100.5	4432.3	66.6	99.9	4.0	75.0	2156.1	46.4	113.7
NFW	0.2	2.2	1.6	1.3	111.9	0.0	0.9	0.3	0.5	95.7
NDW	0.0	0.3	0.0	0.2	117.2	0.0	0.1	0.0	0.1	95.5
LT	24.5	29.3	19.5	4.4	16.7	24.1	30.0	26.8	5.2	19.2
NP	11.5	15.5	8.4	2.9	21.5	7.3	12.8	8.0	2.8	27.7
PL	8.7	10.4	1.0	1.0	10.5	9.2	10.1	0.2	0.5	5.0
GPo	3.2	5.0	1.1	1.1	25.4	3.9	4.7	0.2	0.5	11.4
GPl	40.0	71.5	403.8	20.1	36.1	33.5	50.8	157.2	12.5	28.8
GDW	9.0	16.6	21.6	4.7	35.9	7.5	11.0	7.6	2.8	30.1

The CV values were lower for reproductive traits and leaf temperature than for vegetative traits. In all cultivars and irrigation conditions, pod length showed the smallest variation, from 5.0% to 10.5%, whereas the greatest variation was seen for the traits related to nodulation, such as number of nodules, which ranged from 78 .9% to 113.7%. This result suggests that a larger sample size was needed to estimate the mean number of nodules than pod length. The CV values were similar to those found for grains per pod and lower than those found for number of pods and grains per plant in common bean (Cargnelutti et al., 2008). The CV values were also higher than those found for plant height at maturity and number of nodes per plant and lower than those found for number of pods in soybean (Cargnelutti et al., 2009).

The sample size needed to estimate the response variables of the cultivars under each irrigation condition showed greater variability in the 10% confidence interval of the estimate of the mean, in which there is greater experimental precision (Table 3). At a 10% error of the estimate of the mean, >1000 plants are needed. From a practical point of view, there are difficulties in evaluating >1,000 common bean plants. Bandeira et al. (2018) observed the same in rye (920 plants at 5% error of the estimate of the mean) and stressed the acceptance of larger estimation errors to provide researchers with greater flexibility in sampling.

Table 3
Sample size, in number of plants, to estimate the mean traits of common bean cultivars (Cv) Triunfo, Garapiá, and FC104 under irrigation conditions (IC: irrigated [I] and non-irrigated [NI]) for confidence intervals lower than 10, 15, 20, 25, 30, 35, and 40% of the estimate of the mean.

Cv	IC	10%	15%	20%	25%	30%	35%	40%
				Height				
Triunfo	I	372	162	91	61	40	31	23
Triunfo	NI	375	157	89	56	37	28	22
Garapiá	I	449	199	117	72	49	36	30
Garapiá	NI	544	243	128	85	58	42	36
FC104	I	408	189	100	65	47	25	32
FC104	NI	407	178	96	63	44	32	25
				Number of nodes				
Triunfo	I	182	82	46	28	20	15	11
Triunfo	NI	178	79	45	29	20	15	12
Garapiá	I	161	75	38	25	17	13	10
Garapiá	NI	152	66	37	24	16	12	9
FC104	I	197	85	49	31	21	16	12
FC104	NI	201	85	47	29	22	17	13
				Stem diameter				
Triunfo	I	142	59	35	22	16	11	8
Triunfo	NI	158	73	38	25	18	13	10
Garapiá	I	99	45	25	15	11	8	6
Garapiá	NI	111	47	26	18	12	9	7
FC104	I	91	40	22	15	10	7	6
FC104	NI	62	31	15	11	8	6	4
				Root length				
Triunfo	I	269	117	63	39	29	22	15
Triunfo	NI	266	114	67	40	28	21	15
Garapiá	I	193	90	50	32	20	17	12
Garapiá	NI	205	95	50	32	23	17	13
FC104	I	142	62	36	21	16	12	9
FC104	NI	137	64	35	22	15	11	9

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Shoot fresh weight								
Triunfo	I	673	292	172	105	77	56	39
Triunfo	NI	600	248	136	88	64	41	34
Garapiá	I	532	240	134	84	62	34	44
Garapiá	NI	418	182	103	66	45	32	25
FC104	I	550	235	135	85	57	43	32
FC104	NI	607	268	145	94	65	45	36
Shoot dry weight								
Triunfo	I	697	305	168	112	71	54	42
Triunfo	NI	650	283	151	98	73	48	36
Garapiá	I	728	340	173	110	78	59	42
Garapiá	NI	475	212	127	78	52	44	32
FC104	I	619	254	145	91	64	47	36
FC104	NI	538	233	122	81	59	43	32
Root fresh weight								
Triunfo	I	837	366	203	143	92	66	50
Triunfo	NI	736	317	189	114	78	61	43
Garapiá	I	664	290	160	103	69	52	40
Garapiá	NI	754	341	196	123	81	59	47
FC104	I	714	324	176	114	77	55	46
FC104	NI	>1000	518	283	181	126	92	72
Root dry weight								
Triunfo	I	776	328	175	113	75	58	44
Triunfo	NI	>1000	461	254	170	109	80	60
Garapiá	I	729	328	189	123	78	60	43
Garapiá	NI	799	330	187	116	85	63	46
FC104	I	813	355	199	126	95	65	51
FC104	NI	860	378	208	136	89	66	49
Number of nodules								
Triunfo	I	>1000	834	480	288	199	144	112
Triunfo	NI	>1000	493	268	172	118	86	64
Garapiá	I	>1000	750	411	279	189	137	106
Garapiá	NI	>1000	458	244	161	111	80	60
FC104	I	>1000	755	401	252	168	124	98
FC104	NI	>1000	917	497	317	218	163	122
Nodule fresh weight								
Triunfo	I	>1000	748	416	272	184	137	101
Triunfo	NI	938	435	223	146	102	72	57
Garapiá	I	>1000	609	329	215	147	111	78

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Garapiá	NI	637	282	157	96	67	50	38
FC104	I	>1000	914	486	310	217	162	124
FC104	NI	>1000	529	360	234	148	114	85
Nodule dry weight								
Triunfo	I	>1000	974	522	343	225	179	121
Triunfo	NI	949	427	230	151	104	75	56
Garapiá	I	>1000	592	334	211	144	106	82
Garapiá	NI	823	359	207	127	94	62	51
FC104	I	>1000	974	554	336	235	168	132
FC104	NI	>1000	611	361	222	153	114	84
Leaf temperature								
Triunfo	I	38	17	10	6	4	3	3
Triunfo	NI	63	26	14	10	7	5	4
Garapiá	I	53	24	14	9	6	5	4
Garapiá	NI	71	33	18	11	8	6	5
FC104	I	43	20	12	7	5	4	3
FC104	NI	67	28	17	11	8	6	5
Leaf area								
Triunfo	I	332	144	85	52	38	26	21
Triunfo	NI	138	65	34	22	17	12	9
Garapiá	I	441	198	107	69	50	34	25
Garapiá	NI	413	188	100	66	44	34	25
FC104	I	634	271	161	93	68	51	37
FC104	NI	327	145	78	52	35	26	20
Number of pods								
Triunfo	I	80	34	20	13	9	6	5
Triunfo	NI	76	33	18	12	8	6	4
Garapiá	I	100	11	25	15	11	8	6
Garapiá	NI	96	44	24	16	10	8	6
FC104	I	69	32	18	12	8	6	5
FC104	NI	115	50	28	17	13	9	8
Pod length								
Triunfo	I	12	5	3	2	2	2	2
Triunfo	NI	8	4	2	2	2	2	2
Garapiá	I	13	6	4	2	2	2	2
Garapiá	NI	10	5	3	2	2	2	2
FC104	I	16	7	4	3	2	2	2
FC104	NI	4	2	2	2	2	2	2

continue...

continuation...

Grains pod^{-1}								
Triunfo	I	35	16	9	6	4	3	3
Triunfo	NI	59	24	14	9	7	5	4
Garapiá	I	55	24	14	9	7	5	4
Garapiá	NI	49	24	12	9	6	4	3
FC104	I	96	43	26	16	11	8	7
FC104	NI	20	9	6	4	3	2	2
Grains plant^{-1}								
Triunfo	I	132	55	31	20	15	11	8
Triunfo	NI	82	37	19	13	9	7	5
Garapiá	I	84	36	20	14	9	7	5
Garapiá	NI	81	35	20	12	9	79	5
FC104	I	197	84	50	30	21	17	12
FC104	NI	126	55	31	20	14	10	8
Grain dry weight								
Triunfo	I	144	62	34	23	16	11	9
Triunfo	NI	99	44	25	16	10	9	6
Garapiá	I	87	39	22	14	10	7	6
Garapiá	NI	90	41	24	14	11	8	6
FC104	I	195	82	46	32	22	16	11
FC104	NI	141	62	34	24	16	11	8

At a confidence level of 95% and an error of 40% of the estimate of the mean, the sample size to evaluate all the analyzed traits is 132 plants, a value close to the 114 plants found for jack bean (Cargnelutti et al., 2018b) and higher than the 42 plants found for rosemary seedlings (Schoffel et al., 2019). Therefore, under the conditions in which the experiments were conducted, 132 plants are required to determine the mean of the 18 traits measured. Accordingly, in an experimental design with four replicates, 33 plants should be evaluated per experimental unit in each treatment. In practice, this still entails difficulties due to the high number of plants, so we suggest using the largest number of samples necessary from the traits measured in each experiment, thus reducing the sample size. Considering all cultivars and irrigation conditions evaluated, the number of plants needed were: 36 for height, 13 for number of nodes, 10 for stem diameter, 15 for root length, 44 for shoot fresh weight, 42 for shoot dry weight, 72 for root fresh weight, 60 for root dry weight, 122 for number of nodules, 124 for nodule fresh weight, 132 for nodule dry weight, 37 for leaf area, 5 for leaf temperature, 8 for number of pods, 2 for pod length, 7 for grains per pod, 12 for grains per plant, and 11 for grain dry weight. Cargnelutti et al. (2008) evaluated 14 common bean genotypes and found similar mean values: 9 plants for number of pods, 10 for grains per plant, and 2 for grains per pod. The authors concluded that a sample of 10 plants is satisfactory to evaluate reproductive traits a value that is close to the 12 plants found in the present study.

Corroborating the lower CV values (Table 2), the reproductive traits and leaf temperature required a smaller sample size than the vegetative traits. This is in disagreement with what was found for turnip, where the sample size for yield traits was larger than for morphological traits (Cargnelutti et al., 2014). The exception among vegetative traits was stem diameter. In pigeon pea, stem diameter showed low variability, as its homogeneity increases throughout the crop cycle (Facco et al., 2015).

Within each combination of cultivar vs. irrigation condition, with the exception of shoot fresh weight, higher CV resulted in larger sample sizes (Tables 2 and 3). The same was also observed by Kleinpaul et al. (2017), Cargnelutti et al. (2018b), and Schoffel et al. (2019, 2020). Therefore, considering the same experimental precision for the estimation of the mean of common bean traits, the sample size determined by the bootstrap resampling method differs between traits and between the cultivars and irrigation conditions used. This variability in sample size denotes that the results are adequate, as they reflect real experimental situations. The same was found for millet (Kleinpaul et al., 2017), flax (Cargnelutti et al., 2018a), jack bean (Cagnelutti et al., 2018b), rosemary (Schoffel et al., 2019), and cassava (Schoffel et al., 2020).

The root-related traits exhibited great variability (Table 2) and required large sample sizes (Table 3). Gonçalves et al. (2017) highlighted that studying root traits is complex and difficult, even more so in soil cultivation. Soil cultivation, even in pots, compromises the total evaluation of plant roots. In rosemary seedlings with a substrate composed of 100% soil, Schoffel et al. (2019) observed high CV values for root dry weight and root length: 54.65 and 46.31%, respectively. The greatest variability in CV and sample size among the studied traits occurred in those related to nodulation (number of nodules, nodule fresh and dry weights). The higher CV is probably associated with natural variability caused by nodulation. However, the observed CV are higher than those reported in other studies with common bean, i.e., from 20.32 to 22.04% for number of nodes and 20.16 to 26.10% for nodule dry weight (Carvalho et al., 2020) and 20.5% for number of nodules (Shumi et al., 2018). This can be explained by the fact that, in the present study, the data were collected throughout the plant growth and development cycle, which causes greater variability, resulting in a need to measure a greater number of plants than in other traits. Therefore, determining the number of samples from data throughout the common bean cycle for root traits may not be the most appropriate approach, but rather from data at phenological stages. In pigeon pea, Facco et al. (2015) observed variability in sample size between crop development stages, e.g., in plant height at the beginning of the crop cycle, which required a larger sample size, due to variability in the observations, than at the end of the crop cycle. In rye, Bandeira et al. (2018) also observed a difference in sample size at different evaluation times.

Disregarding the CV of the nodulation traits, which were high, the traits determined by counting showed a lower mean, of 25.78% for all cultivars and irrigation conditions, whereas those obtained by measuring averaged 43.61%, suggesting that, for the same level of experimental precision, more samples are needed for the measured traits than for the counted ones. Schoffel et al. (2020) observed the same result in cassava in an experiment evaluating vegetative traits.

This study provides knowledge input on the number of samples for 18 traits in three common bean cultivars under two irrigation conditions. In both the irrigated and non-irrigated conditions, cultivar Garapiá showed the same sample size for leaf area, number of pods, pod length, grains per plant, and grain dry weight. For Triunfo and FC104, in both irrigation conditions, pod length and root length required the same sample size. Therefore, the sample size for pod length is independent of the irrigation condition. For all other evaluated traits, irrigation resulted in a larger sample size for the three cultivars.

The use of greater experimental precision, adopting a confidence interval of 95% with an error of up to 15% of the estimate of the mean, makes its application difficult due to the high number of plants needed, except for the traits of leaf temperature and pod length, which require 36 and 7 plants sampled, respectively. The information provided in Table 3 allows the user to determine the number of samples based on the size of the experimental area, manpower, financial resources, and experimental precision required.

Conclusions

For cultivars Triunfo, Garapiá, and FC104 in irrigated and non-irrigated conditions, adopting a confidence interval of 95% of error of 40% of the estimate of the mean, 44 plants are needed to evaluate all shoot traits (height, stem diameter, number of nodes, shoot fresh and dry weights, leaf area, and leaf temperature), 132 plants for root traits (root length, fresh and dry weights of roots and nodules, and number of nodules), and 12 plants for yield traits (number of pods, number of grains per pod, grains per plant, pod length, and grain dry weight). Therefore, 132 plants are required to analyze all the studied traits.

Acknowledgements

We thank the Department of Agricultural Research and Brazilian Agricultural Research Corporation for making seeds available and the Coordination for the Improvement of Higher Education Personnel for financing the research.

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