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A High Plant Density and the Split Application of Chemical Fertilizer Increased the Grain and Protein Content of Cowpea (*Vigna unguiculata*) in Burkina Faso, West Africa

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Abstract: In West Africa, cowpea (*Vigna unguiculata*) is an important source of protein for many people. To meet the protein demands, the cowpea yields per unit area and its seed protein content must be increased. We evaluated the effects of the planting density and topdressing (fertilisation) timing on the cowpea yield and its protein content. High density (HD: 40 × 40 cm) and super high density (SHD: 40 × 20 cm) plantings were the most efficient approaches for enhancing cowpea yields. Across different regions in Burkina Faso, under such approaches, the yield significantly increased by as much as 214.5%, with an average value of 88.9%. Fertilisation was not required to achieve the significant increases in cowpea production following dense planting. Although the yield increased, the seed weight per plant decreased with the increase in planting density. Applying topdressing when the plants started flowering increased the seed protein content significantly by up to 24.4%. Simple and robust technologies, such as high-density planting and topdressing, can be rapidly disseminated for increased cowpea yields and protein content improvement for Burkina Faso and for other countries.

Keywords: legumes; cowpea; fertilization; seed yield; planting density; seed protein content

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1. Introduction

Legumes are important sources of protein and other nutrients, and they are vital for satisfying the increasing demands for food and feed. Legume crops can provide multiple services, contributing to sustainable food production. In addition to serving as a fundamental and worldwide source of high-quality food and feed, legumes contribute to reducing greenhouse gas emissions, as they release 5–7 times less greenhouse gases per unit area compared with other crops [1]. Legume farming can also result in carbon sequestration in soils, with values estimated at 7.21 g kg^{-1} of dry matter, with 23.6 C kg^{-1} versus 21.8 g C kg^{-1} per year, and can reduce fossil fuel energy inputs compared with cereal crops due to their lower requirement for N fertilizer, corresponding to 277 kg ha^{-1} of CO_2 per year [1]. Cowpea (*Vigna unguiculata*) is an annual legume grown extensively in Africa, the Americas, Asia, and Europe. It is of vital importance to the livelihoods of millions of people in West Africa.

Cowpea is a starchy legume that complements cereal-rich diets. West Africa accounts for more than 80% of its annual world production (7.23 million metric tons [2]), and the harvested cowpea is primarily consumed locally. Nutritionally, dry cowpea grains contain, on average, 23–32% proteins and 50–60% carbohydrates [3]. It is a nutritious and inexpensive source of protein for both rural and urban consumers [4]. Additionally, cowpea fodder is a valuable source of feed for livestock [5], making it attractive to small-scale farmers [6]. Burkina Faso is an important cowpea producer (third largest in production) in West Africa [7]. Although some cowpea farmers in Burkina Faso adapted recent management practices, which are not yet common due to limited budgets [8]. In addition, the prevalence of chronic malnutrition in Burkina Faso remains of concern. According to a nutritional survey conducted in the country in 2016, the national prevalence of chronic malnutrition was estimated to be 7.6% for all men, women and children (Ministry of Health and National Institute of Statistics and Demography (Burkina Faso) [9]). Therefore, there is an urgent need to establish sustainable agriculture strategies in Burkina Faso, along with simple and easy-to-apply technologies that allow local farmers to easily implement these strategies and/or techniques. Such farming strategies for the use of farmers' preferred crops are indispensable for Africa's farming future. The high-protein content in cowpea can help combat malnutrition; thus, cowpea has an important role in the food security for millions of people [10], including the most economically depressed communities in developing countries [11].

International and national cowpea breeding programs have developed and released various improved varieties for their adoption in cowpea production in Africa. High-protein cowpea breeding has been conducted in West Africa since 1975 [12]. However, high-protein cowpea is yet to be released. Grain proteins are considered a polygenic trait influenced by environmental and management factors [13]. The main genetic loci identified for protein content and seed size are highly heritable; therefore, these traits can be improved in cowpea (with other favourable agronomic traits) through breeding [14,15]. In cowpea, the single-seed weight and grain crude protein content range from 0.13 to 0.30 g and 23% to 33%, respectively [16]. Within the same plant, the single-seed weight and protein content can vary by up to 0.1 g and 10%, respectively [13]. Therefore, breeding programs that could simultaneously address genotypic averages and reduce intra-plant heterogeneity are required. However, any analysis of polygenes and their application in the breeding or control of environmental factors in farmers' fields is challenging. Conversely, if management practices, such as topdressing, can influence the grain protein content, it would indicate that the protein content can be manipulated in the field. Topdressing (applying additional fertilizer while the crop is growing) when rice, soybean, or wheat are at grain maturity can increase the seed nitrogen content [17–19]. However, it remains unknown if this is also the case for cowpea.

Although the potential yield of improved cowpea varieties is between 3000 and 4000 kg/ha, the current average yield in West Africa is only 450 kg/ha [20]. Therefore, the most persistent challenge to expanding the production and consumption of cowpea is through closing the 'yield gap'. For example, cowpea production in northern Nigeria generally uses wide rows (75 cm apart). This may be because, in Nigeria, the equipment used for ridging is the same as that used for other grain crops, such as maize, soybean, sorghum, and millet. Additionally, spacing and density can vary depending on whether cowpea is the sole crop, or whether it is intercropped. The low density resulting from wide row spacing usually leads to low yields in grain legume crops such as cowpea in West Africa [21]. Kamara et al. [22] reported that the plant density is an important component of the yield of some grain crops (such as cowpea and soybean), and it is important to determine the optimum plant densities for different areas and varieties as they have different growth rates. Therefore, adopting the high-density planting method for cowpea can improve the yield and protein production per unit area.

This study aimed to establish a simple method for increasing the cowpea yield and protein content of seeds. The effects of the planting density and topdressing timing on cowpea yield and the seed nitrogen content were investigated. Our findings could contribute to the achievement of stable and sustainable food production in West Africa.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The varieties, IT99K-573-2-1 and IT98K-205-8, were obtained from the International Institute of Tropical Agriculture (IITA) cowpea breeding unit and KVx442-3-25 was obtained from the Institut de l'Environnement et de Recherches Agricoles (INERA) cowpea breeding program. Specimens were grown in an experimental field at the INERA, Saria station, Koudougou, Burkina Faso (12°17′ N, 2°09′ W). The three varieties were grown under different planting densities and fertilizer treatments, as described in Section 2.2. Each plot measured 3.2 × 3.2 m and was established in a complete randomised block design with three replications. Weed control was manually undertaken two times. Insecticides were sprayed for insect control at approximately 30 days after sowing ('Pacha 25EC': 15 g/mL lambda cyhalothrin and 10 g/L acetamiprid) and at approximately 45 days after sowing ('K-Optimal 250 mL': 15 g/L lambda cyhalothrin, 20 g/L acetamiprid). No symptoms of water stress or nutrient deficiency were evident during the growing period. Cultivation was repeated for three years (2018–2020). The annual effective rainfall (June to October) was 743.7 mm, 632.0 mm, and 914.2 mm for 2018, 2019, and 2020, respectively.

2.2. The Effect of Planting Density and Fertilization on Seed Yield and Weight per Plant

The following four planting density treatments were established: (1) normal density (ND, 40 cm between plants and 60 cm between rows); (2) high density (HD, 40 cm between plants and rows); (3) super high density (SHD, 40 cm spacing between plants and 20 cm spacing between rows); and (4) hyper high density (HHD, 20 cm between plants and rows). Chemical fertilizer (a ratio of nitrogen, to phosphorous, to potassium = 15:15:15) was applied four weeks after sowing (4WAS) for the HD and SHD plots ('HD + fertilizer', 'SHD + fertilizer'). The amount of fertilizer applied was indicated in kilograms per hectare. The sampling and analyses were repeated over three years (2018–2020).

Cowpea seeds were harvested after full maturity, and harvested pods were dried under sunlight for a week. After threshing, the seed weight was measured. Differences among treatments were determined using the Dunnett's test using JMP 5.0.1a (SAS Institute, Cary, NC, USA). In all analyses, a probability value of less than 0.05 was considered statistically significant ($p < 0.05$).

2.3. The Effect of Planting Density at Different Locations

The effect of the planting density on the cowpea yield in farmers' fields were also investigated in three different regions of Burkina Faso for two years (2019 to 2020). The plot sizes and treatments were similar to those in Section 2.1. Three planting density plots (normal, high density, and super high density) were established, as in Section 2.2. The regions were characterised by their annual rainfall. The north region was semi-arid with an annual rainfall of 520 mm, the central region was semi-arid with an annual rainfall of 780 mm, and the south region was sub-humid with an annual rainfall of 1,175 mm. The fertilizer was added at a rate of 100 kg/ha at 4WAS, except in the control plot.

Around the experimental field, the project staff randomly visited cowpea fields and measured the planting density. After that, we contacted 25 cowpea farmers in each village. At the harvest period, a part of cowpea seeds in the farmers' fields were harvested by project staff (1 m × 1 m^2), and the seed weight was recorded. The cowpea yield of farmers' fields was estimated using the seed weight. Differences among treatments were determined using the Dunnett's test using JMP 5.0.1a (SAS Institute, Cary, NC, USA). In all analyses, a probability value less than 0.05 was considered statistically significant ($p < 0.05$).

2.4. The Effect of the Topdressing Timing and Evaluation on the Seed Nitrogen/Protein Content

Plant materials, the experimental location, and the treatments were similar to those in Sections 2.1 and 2.2. The following seven timings of the fertilization treatments were established: (1) control (no chemical fertilizer applied); (2) fertilizer applied four weeks after sowing (4WAS); (3) fertilizer applied eight weeks after sowing (8WAS); (4) fertilizer applied immediately after the first flower bloomed in the treatment plot (fertilizer after first flower bloomed); (5) fertilizer applied when 20% of the flowers had bloomed (20% flowering); (6) fertilizer applied when 50% of the flowers had bloomed (50% flowering); and (7) fertilizer applied twice, four weeks after sowing, when 50% of the flowers had bloomed (fertilizer 4WAS + 50% flowering). In all treatments, the fertilizer used was readily available in Burkina Faso (N:P:K = 15:15:15). All treatments received 100 kg fertilizer per ha; in treatment 7, half of this was applied at 4WAS and the other half was applied at 50% flowering.

Cowpea seeds were harvested after their full maturity in 2019 and 2020. After threshing, the seeds were oven-dried at 80 °C for 24 h, and 20 seeds were randomly selected from each treatment. Each selected seed lot was scanned with a Fourier transform infrared spectrometer (FT-IR 6100; JASCO Corporation, Tokyo, Japan) equipped with a reflectance unit (NRF PRO410-N), broadband KBr beam splitter (KBRBB-6000BS), a halogen lamp as the light source, and an InGaAs detector. The spectra acquisition (4000 to 10,000 cm^{-1}) was conducted using the diffuse reflectance method at 8 cm^{-1} resolution with 32 accumulations. To determine the seed nitrogen content, the calibration model following the method of Ishikawa et al. [23] was applied. The seed protein content was calculated by multiplying the nitrogen content by the N:P conversion factor (5.45) and expressing it as a percentage of dry weight, following Muranaka et al. [24].

The differences among treatments with different timings of fertilizer applications were determined using Dunnett's test in JMP 5.0.1a (SAS Institute).

3. Results

3.1. The Effects of Planting Density on Cowpea Cultivation

Figure 1 shows the yield of the three improved cowpea varieties grown under different planting densities and fertilizer treatments. Under ND, the yield of IT99K-573-2-1 was 552.5 kg/ha. Comparatively, the yield of IT99K-573-2-1 under HD and SHD was significantly higher (979.6 kg/ha, and 1014.4 kg/ha, respectively). Although the yield under HD + fertilizer and SHD + fertilizer was higher than the yield under HD and SHD, the differences were not statistically significant. The yields of IT98K-205-8 exhibited a similar pattern. In addition, the KVx442-3-25 yield exhibited a similar pattern; however, it was significantly higher under SHD + fertilizer than under any other treatment (Figure 1). Under HHD, the leaves of each plant overlapped, plant growth was stunted, and there was poor development of pods and seeds. Therefore, the results for the HHD treatment are not reported.

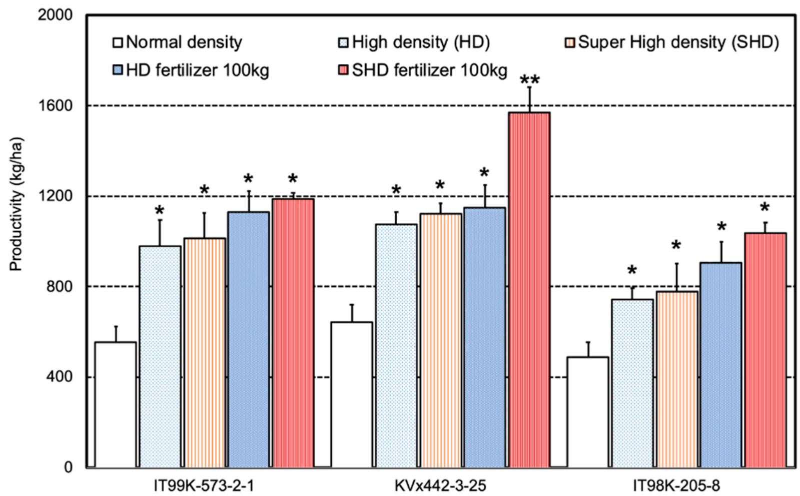

Figure 1. Effect of planting density and fertilizer treatments on cowpea seed production. Bars indicate standard deviation. Asterisks indicate significant differences using the Dunnett's test (* = $p < 0.05$, ** = $p < 0.01$).

The seed weight per plant gradually decreased with the increasing planting density for all varieties (Table 1). For example, the total seed weight of IT99K-573-2-1 under ND was 11.23 g/plant, whereas it was 8.38 and 4.44 g/plant under HD and SHD, respectively. The reduction was approximately 30–40%.

Table 1. Total seed weight per plant with different planting densities.

Variety/Planting Density	Total Seed WT (g/plant)	No. of Plants per Plot (3.2 × 3.2 m)	Estimated Seed WT (kg/ha)
IT99K-573-2-1 normal density	11.23	70	786.0
High density	8.38	144	1206.5 *
Super high density	4.44	266	1180.2 *
KVx442-3-25 normal density	11.24	70	786.9
High density	7.77	144	1118.9 *
Super high density	4.17	266	1108.6 *
IT98K-205-8 normal density	8.07	70	564.9
High density	5.21	144	750.5 *
Super high density	2.78	266	738.8 *

Asterisks indicate significant difference vs. control value through Dunnett's test $p < 0.05$.

3.2. The Effects of the Planting Density on Cowpea Yield in Different Agro-Ecological Zones in Farmers' Fields

Table 2 shows the locations of the multi-location trials, the annual rainfall, the estimated yield, and the planting density of farmers' fields. Farmers used a wider planting density than the experimental fields and the yield range was 400–600 kg/ha. In addition, the farmers used local varieties and they did not use the improved variety.

Table 2. Location of base station and target village/region, basic productivity, and planting density at farmers' fields.

Name of Village	North Latitude	West Longitude	Annual Rainfall (mm)	Estimated Yield (kg/ha)	Planting Density (cm)
Tougouri (north)	13°31′	0°52′	520	594.7	75.7 × 62.0
Pouni (central)	11°95′	−2°54′	780	402.7	64.6 × 52.9
Tiefora (south)	10°63′	−4°55′	1175	415.2	68.9 × 49.1
Saria station (INERA, BF)	12°28′	−2°15′	790	-	60.0 × 40.0

In the northern region, the yield of the three improved varieties varied widely with ND. KVx442-3-25 had a higher yield than IT99K573-2-1 and IT98K-205-8; the yields were 724.2, 460.4, and 355.5 kg/ha, respectively (Table 3). The HD and SHD treatments resulted in an increase in yields of 123.3% and 214.5% compared to ND, respectively. Yields with HD and SHD were 113.8% and 120.2% higher than that of ND in the central region, and were 61.9% and 77.9% higher than that of ND in the southern region, respectively.

Table 3. Effect of high-, super high-density planting for the yield.

Name of Village	Variety and Planting Density	Yield (kg/ha)	Standard Error	Rate of Yield Increase (%)
Tougouri (north)	IT99K573-2-1 ND	460.4	47.5	-
	IT99K573-2-1 HD	876.3 *	63.9	90.3
	IT99K573-2-1 SHD	1167.6 *	83.6	153.6
	KVx442-3-25 ND	724.2	60.8	-
	KVx442-3-25 HD	1097.1 *	126.7	51.5
	KVx442-3-25 SHD	1295.9 *	179.2	78.9
	IT98K-205-8 ND	355.5	39.3	-
	IT98K-205-8 HD	794.2 *	63.9	123.3
	IT98K-205-8 SHD	1118.2 *	95.9	214.5

Table 3. *Cont.*

Name of Village	Variety and Planting Density	Yield (kg/ha)	Standard Error	Rate of Yield Increase (%)
Pouni (central)	IT99K573-2-1 ND	1031.6	143.1	-
	IT99K573-2-1 HD	1916.4 *	176.6	85.8
	IT99K573-2-1 SHD	1889.3 *	204.6	83.1
	KVx442-3-25 ND	656.5	197.3	-
	KVx442-3-25 HD	1279.0 *	144.3	94.8
	KVx442-3-25 SHD	1154.1 *	153.0	75.8
	IT98K-205-8 ND	611.9	39.5	-
	IT98K-205-8 HD	1308.2 *	116.3	113.8
	IT98K-205-8 SHD	1347.6 *	114.9	120.2
Tiefora (south)	IT99K573-2-1 ND	774.6	127.9	-
	IT99K573-2-1 HD	1159.0 *	89.6	49.6
	IT99K573-2-1 SHD	1378.1 *	103.8	77.9
	KVx442-3-25 ND	1079.3	39.0	-
	KVx442-3-25 HD	1499.0 *	86.1	38.9
	KVx442-3-25 SHD	1375.9 *	110.3	27.5
	IT98K-205-8 ND	665.5	127.3	-
	IT98K-205-8 HD	1077.7 *	100.3	61.9
	IT98K-205-8 SHD	1056.9 *	130.7	58.8

ND: normal density, HD: high density, SHD: super high density. Asterisks indicate significant difference vs. control (ND) value using Dunnett's test $p < 0.05$.

3.3. The Effects of Topdressing Timing on Cowpea Protein Content and Yield

Compared with the control, fertilizer treatments increased the cowpea yield (Table 4). However, this increase was not statistically significant under 8WAS for both IT99K-573-2-1 and IT98K-205-8. The 'after first flower bloomed' treatment resulted in a significantly higher yield compared with the controls for all varieties. For KVx442-3-25, all fertilizer treatments significantly increased the yield compared with the control. Conversely, the seed protein content was only significantly higher than that of the control under the 'fertilizer after first flower bloomed' treatment.

Table 4. Productivity and protein content under different timings of fertilizer application.

Fertilizer Treatment	IT99K-573-2-1			KVx442-3-25			IT98K-205-8		
	Productivity kg/ha	SE	Protein Content (%)	Productivity kg/ha	SE	Protein Content (%)	Productivity kg/ha	SE	Protein Content (%)
Control	552.5	73.2	16.2	644.0	76.3	12.8	488.0	66.5	15.6
Fertilizer 4WAS	1238.7 *	125.1	17.0	1165.9 *	80.1	13.1	835.0 *	68.1	15.6
Fertilizer 4WAS + 50% flowering	1109.1 *	130.4	14.9	1116.3 *	77.2	13.0	505.4	38.7	15.0
Fertilizer after first flower bloomed	1150.5 *	90.5	18.1 *	1064.2 *	90.6	15.6 *	837.8 *	69.5	17.2 *
Fertilizer 20% flowering	1180.9 *	94.7	16.7	1113.4 *	63.0	13.4	720.3	89.1	15.3
Fertilizer 50% flowering	1054.2 *	82.9	16.7	934.0 *	61.8	13.5	826.3 *	83.9	15.2
Fertilizer 8WAS	935.6	114.0	15.4	976.5 *	87.3	13.4	629.0	49.4	15.9

SE indicates the standard error. Asterisks indicate significant difference vs. control value using Dunnett's test $p < 0.05$.

3.4. The Effects of Topdressing at the Early Flowering Period on Cowpea Protein Content

Table 5 shows the effects of topdressing after the first flowers bloomed on the cowpea seed protein content. Under the control treatment, the protein contents of IT99K-573-2-1, KVx442-3-25, and IT98K-205-8 were 16.84%, 13.08%, and 16.81%, respectively. The protein content was significantly higher with topdressing treatment, compared to the control, for all varieties in both years. The protein contents were 20.1% (IT99K-573-2-1), 16.35%

(KVx442-3-25), and 19.82% (IT98K-205-8). The highest increase in protein was 24.4% in 2019 (Table 5). The protein content varied with the variety and the year.

Table 5. Effects of topdressing (additional fertilizer) treatment at flowering period for protein content (%) in cowpea seeds.

Variety	2019			2020		
	Control	Topdressing after First Flowers Bloomed	Rate of Increasing Protein (%)	Control	Topdressing after First Flowers Bloomed	Rate of Increasing Protein (%)
IT99K573-2-1	16.84	20.11 *	19.4	16.19	17.77 *	10.0
KVx442-3-25	13.08	16.35 *	24.4	12.81	14.99 *	17.1
IT98K-205-8	16.81	19.82 *	17.9	15.59	17.11 *	9.1

Asterisks indicate significant differences vs. control value using Dunnett's test $p < 0.05$.

4. Discussion

Plants often compete with each other after their emergence; therefore, the planting density is often adjusted to maximize the yield [25–30]. The planting density is particularly important for the growth of grain legumes, such as cowpea and soybean [22]. Jallow and Fergusson [31] and Kwapata and Hall [32] reported that the effects of plant density on the cowpea seed yield were consistent with those for soybean. Generally, yields do not increase linearly with an increase in plant density due to interplant competition [22,28,33]. Our results support these previous findings. Therefore, the cowpea seed yield was limited by the seed weight per plant. The seed weight per plant is an easy-to-use indicator for the yield. The seed weight and/or yield per plant can be easily determined through observation. Therefore, this indicator can be used to evaluate and disseminate results at local institutes or farmers' fields.

The cowpea varieties responded differently to planting density in the different areas. The improved varieties, IT99K573-2-1 and IT98K-205-8, are extra-early maturing varieties. They are recommended for areas of the Sahel, to semi-arid regions, to avoid the adverse effects of terminal drought [34]. The extra-early maturing characteristics may have contributed to the higher yield of these varieties under HD and SHD, as their yield was higher in the north and central regions. In addition, KVx442-3-25 with the SHD treatment recorded higher yields using the fertilizer application compared to other varieties. These results provided important information on the suitability of these varieties under different conditions and their different responses to fertilizer applications. Furthermore, the findings suggest that the cowpea yield can be easily improved, even in the case of farmers with no access to fertilizer. The results demonstrate the effectiveness of HD and SHD planting in different agro-ecological zones in Burkina Faso. Even if the cowpea yields for any individual farmers do not improve dramatically, the effects of HD and SHD planting could have a large impact on national or regional production.

The fertilizer application is primarily used to increase the grain yield, but it can also be used to increase the seed protein content. The grain nitrogen content can be increased using nitrogen topdressing during the ripening period in rice [17,35,36], soybean [18,37,38], and wheat [19,39,40]. In cowpea, the seed protein content drastically increases during the pod elongation period, especially after the full pod elongation [41]. This suggests that the best time for fertilizer application is before and after the flowering period. As we expected, the seed protein content was most affected by topdressing during the flowering period. Topdressing increased the seed protein content for all three cowpea varieties; however, varietal differences were observed. When farmers purchase and apply fertilizer, they expect higher yields, and they may not consider the potential increase in grain protein content. Therefore, fertilizer application could be a strategy to achieve an increased yield and an increased protein content simultaneously. On the other hand, NPK fertilizer is easy to buy for small-scale farmers and is always available in Burkina Faso. However, the fertilizer applied is a compound and is not appropriate for topdressing because the efficiency of the less soluble nutrients, especially phosphate, is greatly reduced. Topdressing during the flowering period is a simple and robust method of increasing the cowpea seed protein content and yield. Moreover, due to the limitation of the research budget, soil analyses were not performed in this study. The soil fertility and the soil type of the target filed is also important for cowpea yield and/or fertilizer application [42]. However, the soil fertility and soil types are distributed in "mosaic" patterns in Burkina Faso and the Sudan Savanna area [42]. Therefore, it is also important that the selection of genotypes improve the long-term stable yields across diverse soil types in the country.

Although a high protein content in grains is desirable from a nutritional perspective, excessive N topdressing may lead to several problems, such as lodging, a reduced yield, a lower quality, and altered taste, as well as changes in the grain cooking properties [9,40,43]. Furthermore, the optimal rates of N for the quality of the grain proteins across the different cowpea-growing agroecologies in the region need to be determined. Therefore, it may be necessary to conduct a sensory evaluation of the taste of high-protein cowpea with consumers.

5. Conclusions

In Burkina Faso, the utilization of cowpea, which is a familiar and traditional crop, is important for technology dissemination and sustainable agricultural practices. Moreover, it is important that the management practices can be easily disseminated and implemented. In this study, we demonstrated that high-density planting and topdressing during flowering can increase the yield and grain protein content of cowpea. High-density planting increased the cowpea yield by 88.9% and topdressing during flowering increased the seed protein content by 24.4%. Of course, it is necessary to evaluate the soil type, the soil fertility, and the varieties of cowpea the farmers use in their locations to adapt to the developed techniques widely. Even so, these simple/easy management practices could improve the income and nutritional status of populations, via increased protein intake, in developing countries.

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