

The potential relationship between the number of root nodules and pod yield in cowpea cultivars subjected to water deficit under greenhouse conditions

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ABSTRACT

The relationship between root nodules and pod yield, especially under water stress, requires understanding to guide farmers' choices and optimise yields in water-stressed environments. This study was initiated to explore the correlation between root nodule density and pod yield in four cultivars of cowpea (KVX745-11P, Tiligre, Gourgou, and KVX780-6) under water deficit conditions. Plants were exposed to three levels of water content: 100% field capacity (FC), 60% FC, and 12.5% FC. The water content levels of 60% FC (moderate water deficit) and 12.5% FC (severe water deficit) represented the various degrees of water deficit imposed on the plants from sowing to wilting. A three-block FISHER device was installed in the greenhouse. Data collection involved manually counting the number of root nodules and pods produced per plant at the end of the growth cycle. The results indicate a relationship between the number of nodules and the number of pods in cowpea cultivars subjected to water stress, with a strong correlation coefficient value of over +0.95. cultivar KVX745-11P produced the highest number (15) of nodules, while the lowest number of root nodules was recorded in the Tiligre and KVX780-6 cultivars with one root nodule each, respectively. These findings are beneficial for farmers by guiding them in selecting cowpea cultivars best suited for water-stressed areas while highlighting the importance of root nodules in plants' ability to withstand water stress while maintaining optimal yields.

Keywords: cowpea cultivars, number of nodules, number of pods, relationship, water deficit

INTRODUCTION

Cowpea is an important leguminous crop in various agricultural landscapes due to its resilience to harsh conditions and its crucial role in food security and soil fertility (Rani et al., 2019; Vanlauwe et al., 2019). Root nodules, symbiotic structures formed through interaction between cowpea plants and nitrogen-fixing bacteria, play a critical role in enhancing nitrogen availability, thereby promoting plant growth and productivity (Bano and Iqbal, 2016; Gopalakrishnan et al., 2015). Additionally, cowpea

exhibits remarkable tolerance to water deficit, making it a favorable crop in water-stressed regions (De Souza Silva et al., 2024). Given the increasing water scarcity, understanding the interaction between root nodulation and yield becomes imperative for sustainable agricultural production (Dutta et al., 2022). The ability of legumes to form root nodules through symbiotic interactions with nitrogen-fixing rhizobia is well-documented. Nodules serve as sites for biological nitrogen fixation,

facilitating nitrogen supply to plants and enhancing soil fertility (Lindström and Mousavi, 2020). Consequently, any alteration in nodule formation or function can significantly influence plant growth, development, and ultimately, yield (Aranjuelo et al., 2014).

Cowpea is of paramount importance in sustainable agriculture, food security, and human health (Kebede and Bekeko, 2020). One of the main reasons for its significance lies in its nutritional value, as it is a legume consumed as a source of high-quality plant proteins in many regions of the world (Jayathilake et al., 2018). Additionally, cowpea is rich in proteins, fiber, vitamins, and minerals, making them an excellent source of essential nutrients, particularly in regions with limited access to diverse diets (Abebe and Alemayehu, 2022).

Water deficit is one of the primary challenges faced by agricultural systems. It exerts profound effects on plant physiology, particularly on root morphology, water use efficiency, and nutrient absorption (Bhattacharya, 2021). Although the impact of water deficit on cowpea yield has been extensively studied, its specific influence on root nodule formation and their relationship with pod yield remains less explored. The overarching objective of this study was to investigate the correlation between root nodule density and pod yield in different cowpea cultivars under water deficit conditions. Specifically, this study involved counting the number of root nodules and pods to establish their relationship in cowpea cultivars subjected to water deficit. Such knowledge is crucial for guiding farmers in selecting cowpea cultivars best suited to water-constrained environments. Furthermore, it underscores the crucial role of root nodules in plants' ability to tolerate water stress while maintaining optimal agricultural yields.

MATERIALS AND METHODS

Greenhouse condition and soil field capacity

The study was conducted at Nangui Abrogoua University (5°23'19"N and 4°0'54"W) from January to March and from May to July 2022, within a polyvinyl

greenhouse measuring 10 × 10 × 4 m (L × W × H). The daily temperature inside the greenhouse ranged from 21 °C to 47 °C, with an average of 32.41 ± 4.83 °C. The daily average humidity was 51.52 ± 20.25%, ranging from 20% to 87%. The greenhouse floor consisted of an approximately 8 cm thick layer of sand. The study employed the gravimetric method to determine the soil field capacity (Kramer and Boyer, 1995). Soil samples were collected at a depth of 0 to 25 cm, representing the A horizon of the university forest relic soil. An experimental pot, 25 cm in diameter and 50 cm in height was filled with 6 kg of dry soil. The pot was saturated with water and covered with aluminium foil, then placed on a support to collect excess water after 24 hours of drainage. The volume of water retained in the soil, corresponding to the field capacity, was obtained by subtracting the poured water volume from the collected water volume after 24 hours of drainage. The field capacity of the soil was determined to be 2.08 L (100% FC). The water volume of 2.08 L, corresponding to the soil's field capacity, was reduced to 1.25 L, which is 60% of the field capacity (FC), representing conditions of moderate water stress. The same water volume (2.08 L) was then reduced to 0.25 L, which is 12.5% of the FC, corresponding to severe water stress conditions. The physicochemical characteristics of the university forest relic soil used in the experiment were described by (Vennetier, 1973).

Plant material

The plant material consisted of four cultivars of cowpea provided by the Institute of the Environment and Agricultural Research (INERA) of Burkina Faso. These were K VX745-11P, Tiligre, Gourgou, and K VX780-6 (Figure 1). The characteristics of these cultivars are listed in Table 1.

Experimental design and crop management

The plants were exposed to three levels of water content: 100% field capacity (FC), 60% FC, and 12.5% FC. The water content levels of 60% FC (moderate water deficit), and 12.5% FC (severe water deficit) represented

the various degrees of water deficit imposed on the plants. A three-block FISHER device was installed in the greenhouse. In each block, each treatment was replicated three times. Each block comprised 36 treatments arranged randomly. A treatment represented the combination of a cultivar and a water level. In total, 108 treatments were

arranged in the greenhouse. Each block contained two rows of 18 treatments each. The rows were spaced 0.5 m apart, and the treatments within the rows were spaced 0.20 m apart. A distance of one meter was maintained between the blocks.



KVX745-11P



Tiligre



Gourgou



KVX780-6

Figure 1. Seeds of cowpea cultivars

Table 1. Characteristics of cowpea cultivars studied

Cowpea cultivars	Cycle (day)	Stem	Seeds color	Seeds size
KVX745-11P	70	upright	yellow khaki	small
Tiligre	70	creeping	White	large
Gourgou	75	creeping	White	large
KVX780-6	70	creeping	White	large

Plant watering in pots was performed weekly to induce water deficit conditions (60% FC, and 12.5% FC), while regular watering was applied to plants in pots at 100% FC (control condition) from seeding to plant senescence. Thus, plants subjected to stress at 60% FC, and 12.5% FC received 11.25 L, and 2.25 L, respectively, throughout the nine-week experiment. Pots containing sown seeds were placed on supports to prevent root penetration into the ground.

Data collection

Data collection involved manually counting the number of root nodules and pods produced per plant at the end of the growth cycle. The plant roots were carefully washed to remove all traces of soil. Nodule counting was performed on all of the roots.

Statistical analysis

The data obtained in this study were analyzed using the ANOVA method with the R software (R Core Development Team, 2022). The LSD test was employed to compare mean results, and the level of statistical significance was determined at a probability of 5%. The homogeneity test results showed that there was no significant difference between the two sets of experiments ($P < 0.05$). Consequently, ANOVA was performed by combining the data from both sets of experiments (Levene, 1960). Graphs were constructed using Excel software (Microsoft Office Excel, 2016) based on the means recorded from nine repetitions per treatment.

RESULTS

Relationship between root nodules and pods in the KVX745-11P, Tiligré, Gourgou and KVX780-6 cultivars under water stress conditions

The evolution of the number of root nodules and the number of pods produced by the cowpea cultivar KVX780-11P is depicted in Figure 2. This figure illustrates that as soil moisture content increases, both the number of root nodules and the number of pods produced by the plants increase. From 12.5% field capacity (FC) to 100% FC, the number of nodules and pods produced rises from 15 to 78 and from three to 16, respectively. The correlation coefficient, $r = +0.97$ indicates a very strong positive correlation between the number of nodules and the number of pods. As the number of root nodules increases, the number of pods also tends to increase.

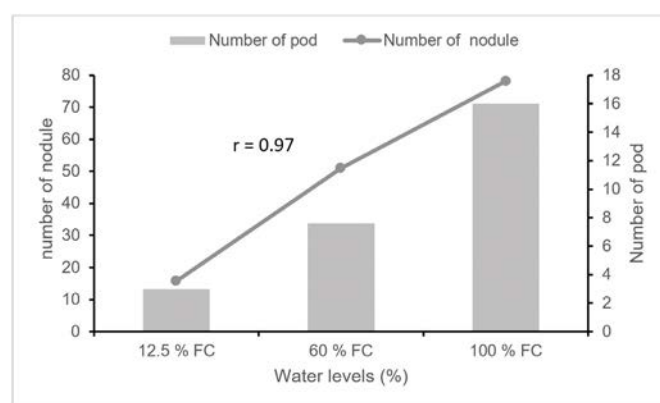


Figure 2. Evolution of root nodule count and pod number in the KVX745-11P cultivar

Figure 3 presents the evolution of the number of nodules and pods produced by Tiligre cultivar plants under water deficit stress. The very strong correlation coefficient value ($r = +0.99$) demonstrates a relationship between nodule production and pod number in the Tiligre cultivar under water deficit conditions (Figure 3). Indeed, under severe water deficit stress (12.5% FC), the number of pods decreases from seven to one, and the number of nodules also decreases from 16 to one.

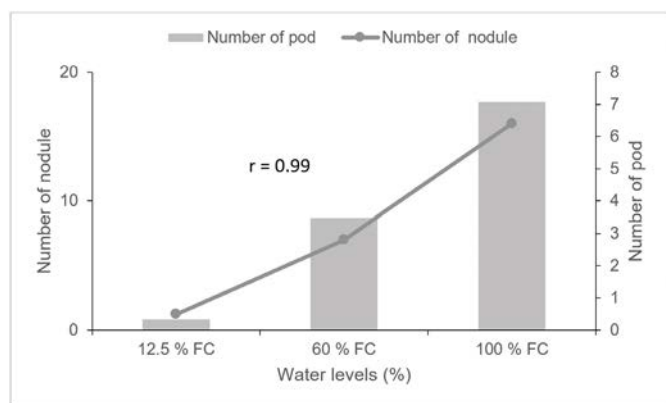


Figure 3. Evolution of root nodule count and pod number in the Tiligre cultivar

The progressive increase in the number of root nodules and pods produced by the Gourgou cultivar is depicted in Figure 4. This illustration reveals that as the level of water deficit decreases, both the number of root nodules and the number of pods produced by the plants increase. Between 12.5% FC and 100% FC, the number of nodules and pods produced evolves from four to 32 and from one to six, respectively.

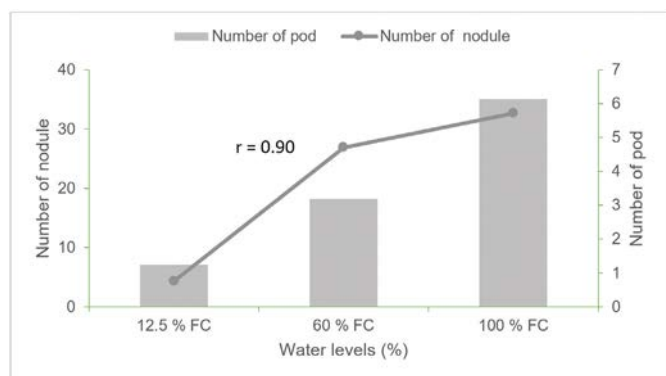


Figure 4. Evolution of root nodule count and pod number in the Gourgou cultivar

The correlation coefficient, established at $r = +0.90$, attests to a very strong positive correlation between the number of nodules and the number of pods under water deficit conditions.

Figure 5 illustrates the evolution of nodule count and pod number in the KVX780-6 cultivar under various water deficit conditions. The very high correlation coefficient ($r = +0.95$) highlights the relationship between nodule production and pod number in this cultivar under water deficit conditions. Indeed, there is observed variation in pod number from one to ten and nodule count from one to 37, transitioning from severe water constraint (12.5% FC) to soil conditions with normal moisture (100% FC).

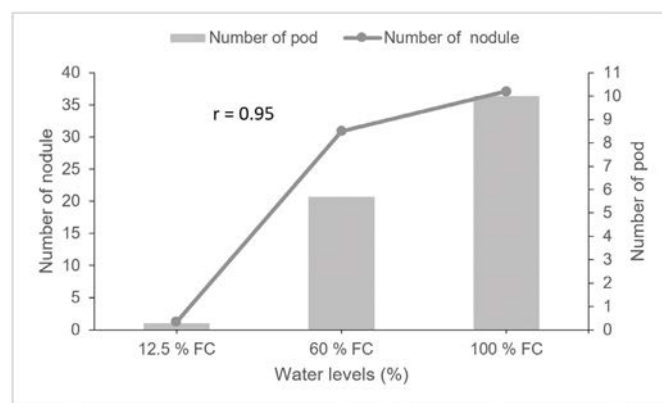
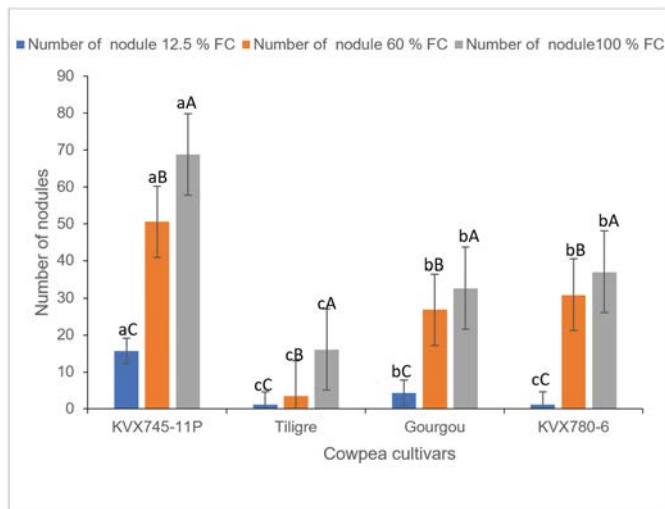


Figure 5. Evolution of root nodule count and pod number in the KVX780-6 cultivar

Comparison of the number of root nodules between cowpea cultivars

The results of the analysis of variance indicate that water deficit levels have a significant effect ($P < 0.05$) on the number of root nodules in all cowpea cultivars (Figure 6). Indeed, the results show that at 100% field capacity (control condition), the KVX745-11P cultivar recorded the highest number of nodules with a value of 68, while the Tiligre cultivar recorded the lowest number of root nodules with a value of 16. Under severe water deficit conditions (12.5% field capacity), the largest number (15) of nodules was obtained in the KVX745-11P cultivar. However, the lowest number of root nodules is recorded in the Tiligre and KVX780-6 cultivars with one root nodule each, respectively. The application of different

levels of water deficit led to a reduction in the number of root nodules in all cultivars.



Lowercase letters are used to classify the four cultivars according to the level of water deficit. Uppercase letters are used to classify the levels of water deficit within each cultivar. They exhibit significant differences ($P < 0.05$) according to the LSD method. $a > b > c$; $A > B > C$.

Figure 6. Number of nodules in cowpea cultivars under water deficit conditions

DISCUSSION

The observed results in the KVX780-11P cultivar indicate a significant decrease in the number of root nodules and the number of pods produced under water deficit stress conditions. The reduction in the number of nodules could be an adaptive response of the plant to reduce energy expenditure in the formation of costly structures under water stress conditions. Similar results were obtained by Maluki (2023) in beans. Drought stress is the most devastating factor, affecting both rhizobium growth and the Rhizobium-legume symbiosis (Naveed et al., 2017). The very strong positive correlation between the number of root nodules and the number of pods produced suggests a close relationship between these two variables. An increase in the number of root nodules can enhance nitrogen availability for the plant, thus promoting better growth and development, leading to an increase in the number of pods produced. The work of Darini and Astuti (2023) highlighted the importance of nodules in plant productivity.

The high correlation coefficient ($r = +0.99$) reveals a close relationship between the number of nodules and the number of pods in the Tiligre cultivar. Under severe water stress, with only 12.5% of field capacity (FC), both the number of nodules and pods decrease. This inverse relationship between the number of nodules and the number of pods under water stress can be explained by the plant's adaptive response to an unfavorable environment. The plant allocates more resources to the formation of root nodules to maximize nitrogen absorption and its own survival under stress conditions (Pueyo et al., 2021). This increased allocation of resources towards nodules may negatively affect pod production, as available resources are redistributed to meet the plant's priority needs. Similar results have been observed in other studies. For instance, Meena et al. (2017) and Rafique et al. (2022) found similar responses in Chickpea under water stress.

The results reveal a very strong positive correlation ($r = +0.90$) between the number of root nodules and the number of pods produced in the Gourgou cultivar under water deficit conditions. This relationship can be explained by the crucial role of root nodules in biological nitrogen fixation (BNF) in legumes. Under water deficit conditions, plants may intensify nodulation to enhance nitrogen assimilation, thereby promoting protein synthesis and the growth of reproductive organs such as pods (Ullah and Farooq, 2022; Soba et al., 2022). Thus, an increase in root nodules can stimulate pod production by providing an adequate nitrogen supply to plants, even under water stress. Similar results were observed in the study conducted by Istanbuli et al. (2022) on chickpeas.

The results highlight the negative impact of water deficit on nodulation in cowpea plants. Variability in nodulation capacity between the KVX745-11P and Tiligre cultivars implies genetic differences in their response to water stress. It is possible that the KVX745-11P cultivar is equipped with more efficient mechanisms for water stress tolerance, enabling it to maintain nodulation and thus ensure better nitrogen fixation and increased productivity even under water stress. Conversely, the Tiligre cultivar appears to be less suited to water stress conditions,

which may compromise nodulation and productivity under such circumstances. Wu et al. (2022) demonstrated that plants respond differently to water deficit stress. Water stress can reduce the activity of nitrogenase, an enzyme essential for nitrogen fixation in nodules, directly impacting nodule formation and maintenance (Zacarias et al., 2004). It also disrupts the hormonal balance in plants, leading to an increase in abscisic acid (ABA) levels (Seki et al., 2007). High concentrations of ABA can directly inhibit root growth and nodule development, while also inducing early senescence of nodules. This premature senescence further reduces the number of nodules and impairs their functionality (Duan et al., 2013; Daryanto et al., 2015).

CONCLUSION

Understanding the interaction between root nodules and pod yield, especially under water stress, is crucial for guiding farmers' decisions and optimizing yields in water-stressed environments. The results of this study indicate a relationship between the number of nodules and the number of pods in cowpea cultivars subjected to water stress, with a strong correlation coefficient value exceeding +0.95. Cultivar K VX745-11P produced the highest number (15) of nodules, while the lowest number of root nodules was recorded in the Tiligre and K VX780-6 cultivars with one root nodule each, respectively. Further research is essential, focusing on the development of more resilient cultivars and the adoption of sustainable agricultural practices.

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