Cornous Biology
Research Article

Effects of *Rhizobium* strains on seed quality of chickpea (*Cicer arietinum* L.) varieties

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Volume: 2, Issue: 2, Pages: 1-8
DOI: https://doi.org/10.37446/corbio/rsa/2.2.2024.1-8
Received: 02 January 2024 / Accepted: 20 March 2024 / Published: 30 June 2024

Background: Ethiopia is one of the largest chickpea producing countries in the world and ranks first in Africa. However, the yield of the crop is low as compared to the potential of the crop to produce up to 5.5 tons ha^{-1}. The scarcity of high-quality seeds and the poor fertility of the soil are major obstacles to the production of chickpea. Thus, the purpose of this study was to evaluate the effects of seed inoculation with *Rhizobium* strains on the seed quality of chickpea varieties.

Methods: Four varieties of chickpea *viz.,* Eshete, Dimtu, Teketay and Local were inoculated with three *Rhizobium* strains (Cp11, Cp17, Cp41) and one control, arranged in factorial combinations, were evaluated in a completely randomized design with four replications.

Results: The interaction of variety and *Rhizobium* strain had a significant effect on the percentage of normal seedlings germination, seedlings shoot length, seedlings root length and seedling vigour index I. The main factors variety and *Rhizobium* strain significantly influenced seedlings' dry weight and seedlings' vigour index II, and speed of germination was significantly influenced by variety.

Conclusion: The current study concluded that the chickpea variety inoculated with Cp17 *Rhizobium* strain produced the highest quality seeds; thus, it is suggested to consider the variety and *Rhizobium* strain to produce high yield and quality seeds in the study area.

Keywords: *chickpea, Rhizobium, seed, seedlings germination, vigour index*

Introduction

According to Anbessa & Bejiga (2002), Ethiopia is regarded as the secondary center of origin for chickpeas and is among the top five countries in the world for producing them. Africa's top producer of chickpeas, the nation is the continent's biggest grower. In the country's central, northern, and eastern highlands, chickpeas are extensively farmed at elevations between 1400 and 2300 meters above sea level, with an annual rainfall of 500 to 2000 millimeters (Anbessa & Bejiga, 2002). In addition to providing a valuable food supply for humans and animals, chickpeas help farmers make a living. Furthermore, the crop has a critical role in preserving soil fertility (Funga et al., 2016), particularly in regions that receive rain and are dry, where N_2 fixation takes place. Furthermore, the crop acts as a rotation crop to diversify agricultural production systems (Shurigin et al., 2015) and releases phosphorus from the soil that is unavailable to other crops in the system (Selvakumar et al., 2012). Ibsa (2013) discovered that, in contrast to no inoculation (1.6 tons ha^{-1}), chickpea yield (2 tons ha^{-1}) rose after inoculation as a result of higher biological nitrogen. As a profitable crop for smallholder farmers, chickpeas are a great source of protein, fiber, complex carbs, vitamins, and minerals.

Many issues contribute to the low chickpea yield in the country, such as the use of low-quality seeds and native cultivars, diseases spread by seeds, and low plant density (Melese, 2005). West Showa is subject to the same restrictions on chickpea production as the rest of the country. Therefore, it is crucial to create and make high-quality seed of improved varieties available, as well as to find suitable and compatible Rhizobia strains, in order to raise chickpea output in West Showa, reduce the need for costly fertilizer applications, and maintain soil fertility. Seed is the most important component in the production of crops. The lack of good seeds limits the productivity and output of smallholder farmers in emerging Sub-Saharan African (SSA) nations. It is anticipated that the seed alone will account for as much as 20% of crop productivity overall.

Studies have been conducted on the use of premium chickpea varieties' seeds that has been infused with *Rhizobium* strains to boost output in West Showa (Mnalku & Mitiku, 2019; Assefa, 2016). The researchers were informed of the need of using improved cultivars, *Rhizobium* inoculation, and NP fertilizer treatment to increase grain output. The use of premium seed is widely recognized as the first step towards creating a quickly growing, vigorous stand and yielding a profitable crop (Eshete, Dimtu, Teketay, and Farmers' cultivar). However, none of the authors examined the impact of NP fertilizer application and *Rhizobium* inoculation on the quality seed production of improved chickpea varieties. In order to increase crop production in the West Showa Zone of the Oromia Region state, it is crucial to identify enhanced varieties that have a specific Rhizobial strain or strains associated with them in order to generate high-quality and high-yield seeds. This research was initiated to achieve the effects of seed inoculation with *Rhizobium* strains on seed quality of chickpea varieties.

Materials and methods

Description of the experimental site

In Ethiopia's Oromia Regional State, the investigation was carried out at the seed laboratory of the Ambo Agricultural Research Centre. The Center is situated 2195 meters above sea level and is located in latitude 08°57'N and longitude 38°07' E. The region experiences mean annual maximum and minimum temperatures of 25.4^{o}C and 11.7^{o}C, respectively, with an average annual rainfall of 1036 mm. The soil type is Vertisol, classified as heavy black clay soil.

Description of experimental materials

The seeds of three chickpea varieties (Eshete, Dimtu and Teketay) produced in 2021 cropping season were collected from Debre Zeit and Ambo Agriculture Research Centers. The three varieties were released by Debre Zeit Agriculture Research. Additionally, seeds of a local chickpea cultivar commonly grown by farmers in the study area were also collected during the same season.

Treatments and experimental design

The treatments included inoculating four chickpea types (three improved and one farmer's cultivar) without inoculating them with any of the three *Rhizobium* strains (CP41 (commercial), CP11 (local), and CP17 (local). As a result, there were 16 treatments in total 4 (varieties) x 4 (inoculations) in a factorial combination. Four replications of a completely randomized design (CRD) were used to arrange the treatments. The substrate for germination was sand. The pure seed fractions that were obtained from the purity test were used for the germination test. From each treatment, four hundred (400) pure seeds will be extracted and split into four repetitions, each containing 100 seeds. On a plastic box with sterilized sand as the medium, each duplicate was sown. At last, on the tenth day of the seedling's planting seedlings were categorized into normal, abnormal, ungerminated seeds, dead seeds and their percentages were calculated.

Data collected from the seed quality test

Standard germination (StG) test: The germination test for chickpea was done at the temperature of 25^{o}C (ISTA, 1996). Thus, the first and second counts will be done at 8 days after planting, respectively. Sand was use as a substrate for germination. The germination test was done from the pure seed fractions obtain from the pure test. Four hundred seed (400) pure seeds have been taken from each treatment, and divide into four replicates of 100 seeds. Each replicate was sown on a sterilized sand medium on plastic box. Finally, at the 10 days of the planting seedling were categorized into normal, abnormal, ungerminated seeds, dead seeds and their percentages were calculated.

$$\text{StG (\%)} = \frac{\text{Total number of normal seedling}}{\text{Total number of seeds planted}} \times 100$$

Seedling Vigour Test: The seed vigour test was determined by measuring the shoot and root length of seedlings. The seedling shoot and root length were measured after the final count of the germination test. Ten normal seedlings were randomly selected from each replicate and shoot length was measured from the point of attachment to the cotyledon. Similarly, the root length was measured. The averages of shoot and root lengths will be computed by dividing the total shoot or root lengths by the total number of normal seedlings measured (Fiala, 1987). Seedling dry weight: The seedling dry weight was measured after the final count of the StG test. Ten randomly selected seedlings from each replicate were selected and placed in envelopes and dried in an oven at 80^{o}C for 24 hrs. The dried seedling was weighed by using a sensitive balance and the average seedling dry weight was calculated.

Vigour index one and Vigour index II: for each treatment, two vigor indices were calculated. Seedling vigor index I was calculated by multiplying the number of normal seedlings with the average sum of shoot and root length and vigor index II was calculated by multiplying the standard germination percentage with mean seedling dry weight.

Mathematically; Vigor Index I (VIG-I) = Average Seedling Length × Normal seedling %; Vigor Index II (VIG-II) =Normal seedling % × Seedling mean Dry Weight

Speed of germination: Speed of germination was calculated from the daily germination records. In case chickpea Speed of germination shall be obtained was 10 days of germination period; the first count will do on the 5th day. Finally, speed of germination was calculated using the following formula which was given by (Fiala, 1987).

$$Speed\ of\ germination = \frac{N1}{C1} + \frac{N2}{C2} + \cdots + \frac{NF}{CF}$$

Where: N1= number of normal seedlings at first count, N2= number of normal seedlings at second count, NF= number of normal seedlings at final count, C1= days to the first count, C2= days to the second count and CF= days to the final count.

Data analysis

The data that will be collected from seed quality test will be subjected to analysis of variance (ANOVA) for CRD factorial Gen Stat 15th edition statistical software package. The mean values comparison will be performed following the significance test results from ANOVA using least significant difference (LSD) at 5% level of probability.

Results

Seed physiological quality test

Effect of variety and *Rhizobium* strain on seed germination and speed of germination

The variety of chickpeas had a major impact on percentage of normal seedlings and speed of germination. The percentage of normal seedling was also highly impacted by the way the *Rhizobium* strain and chickpea variety interacted. However, both percentage of normal seedlings and speed of germination were not significantly influenced by main effect of *Rhizobium* strain. Similarly, proportion of dead seeds and abnormal seedlings was not significantly influenced by the main effect of variety, *Rhizobium* strain and their interaction. The Farmers cultivar had the fastest germination rate (17.8), statistically equal to the quickest germination rates from the Dimtu and Teketay varieties, while the Eshete variety had the slowest germination rate (Table 1).

Table 1. Main effect of variety on speed of germination of chickpea

Variety	SG (No/days)
Eshete	16.13b
Dimtu	17.13a
Teketey	16.95a
Farmers cultivar	17.8a
LSD (0.05)	**0.60**
CV (%)	**4.99**

Mean values within column designated with similar letters are not significant each other at p<0.05, LSD (5%) = Least significant difference at p<0.05 and CV (%) = percentage of coefficient of variation.

Interaction effect of variety and *Rhizobium* strain on normal seedling of chickpea

The main factor variety and the interaction between the two factors had a substantial impact on the normal seedling percentage of chickpea. The Teketay variety, inoculated by the Cp11 strain, had the highest normal seedling percentage (98.75%). It also showed no statistically significant difference from the local variety, inoculated by the Cp11 and Cp41, and the Teketay variety varieties, inoculated by the Cp17 strain. Additionally, it had statistical parity with the normal seedling percentage from the Eshete, Dimtu, and Farmers cultivars, inoculated by Cp17 strains, and no inoculation. The lowest percentage (97.25%) was observed in Teketay Control and Dimtu with Cp11 and Cp41 strains, with no significant difference among them (Table 2).

Table 2. Interaction effects of variety and *Rhizobium* strain on percentage of normal seedlings germination of chickpea varieties

Variety	*Rhizobium* strain	Normal seedling (%)
Eshete	Cp11	97.75bcd
	Cp17	98.00abcd
	Cp41	97.50cd
	Control	98.25abc
Dimtu	Cp11	97.25d
	Cp17	98.00abcd
	Cp41	97.25d
	Control	98.50ab
Teketay	Cp11	98.75a
	Cp17	98.00abcd
	Cp41	97.75bcd
	Control	97.25d
Farmers cultivar	Cp11	98.50ab
	Cp17	98.75a
	Cp41	98.00abcd
	Control	98.75a
LSD (0.05)		**0.95**
CV (%)		**0.68**

Mean values within column designated with similar letters are not significant each other at p<0.05, LSD (5%) = Least significant difference at p<0.05 and CV (%) = percentage of coefficient of variation.

Effect of variety on seedling shoot length, root length and dry weight

According to an analysis of variance, shoot and root length were significantly influenced by both the main effect of variety and the interaction of variety with Rhizobium strain. Additionally, the main effect of *Rhizobium* strains had a considerable impact on both root length and seedling dry weight.

Table 3. Variation of chickpea varieties and *Rhizobium* strains for seedling dry weight

Variety	SDW
Eshete	0.60b
Dimtu	0.64a
Teketay	0.61b
Farmers cultivar	0.57c
LSD (0.05)	**0.018**
R Strain	
Cp 11	0.60ab
CP 17	0.61a
Cp 41	0.61a
Control	0.59b
LSD (0.05)	**0.018**
CV (%)	**4.29**

Mean values within column designated with similar letters are not significant each other at p<0.05, LSD (5%) = Least significant difference at p<0.05 and CV (%) = percentage of coefficient of variation.

The chickpea variety also had a substantial impact on the dry weight of the seedlings, but the interaction between the variety and the *Rhizobium* strain had no significant effect. The primary *Rhizobium* strain effect did not substantially affect shoot length. Farmers cultivar of chickpea had the lowest seedling dry weight (0.57gm), whereas seeds of the Dimtu variety had the highest seedling dry weight (0.64gm). Both the chickpea seeds inoculated with the Cp41 and Cp17 strains registered the maximum seedling dry weight (0.61gm), with the Cp11 strain producing non-significant differences. The chickpea seed without inoculation had the lowest seedling dry weight (0.59gm) (Table 3)

Interaction effect of variety and *Rhizobium* strain on seedling shoot and root length

Shoot and root length was significantly influenced by main effect of variety and interaction of variety with *Rhizobium* strain. Root length was significantly influenced by main effect of *Rhizobium* strain. Eshete variety treated with the Cp17 strain had the longest shoots (11.61 cm), which was statistically equal to the shoots from the Farmers cultivar inoculated with the Cp11, Cp17, and Cp41 strains and the cultivar that had no inoculation. Additionally, there was no significant difference between the Eshete inoculated with Cp41 and the non-inoculation. The shoot length of the Teketay variety, inoculated by Cp17, was the shortest (7.08 cm), and it did not differ statistically from the shoot length of the Dimtu variety, inoculated by Cp11, and the variety not inoculated. The Farmers cultivar, inoculated with the Cp11 strain, had the longest roots (23.27 cm), which was statistically equal to the root length of Teketay and local varieties, inoculated with the Cp17 and Cp41 strains, and not inoculated. Furthermore, there was no significant difference observed between Teketay Vareity inoculated by Cp11 strain. *Dimtu* variety without inoculation had least root length (15.22cm). The Farmers cultivar inoculated with the Cp11 strain had the longest seedlings (34 cm), which was statistically equal to the length of the seedlings from the Farmers cultivar inoculated with the Cp17 strain and the Teketay variety inoculated with the Cp11 strain. The Dimtu variety, which was not inoculated, had the shortest seedlings (22.25 cm) (Table 4).

Table 4. Interaction effects of variety and *Rhizobium* strain on shoot, root length and seedling length of chickpea varieties

Variety	R strain	SL	RL	Seedling length
	Cp11	10.09bc	18.92de	29.25ef
Eshete	Cp17	11.61a	19.80cde	31.5bcd
	Cp41	11.26a	19.44de	30.5cdef
	Control	10.72ab	18.46e	29.5ef
	Cp11	7.16f	19.25de	26.5h
Dimtu	Cp17	7.60ef	19.59de	27.25gh
	Cp41	8.56de	20.50bcde	29fg
	Control	7.13f	15.22f	22.25i
	Cp11	9.59cd	22.82a	32.5ab
Teketay	Cp17	7.08f	21.95abc	29fg
	Cp41	8.15ef	21.99abc	30.25cdef
	Control	8.48e	21.12abcd	29.75def
	Cp11	10.74ab	23.17a	34a
Farmers cultivar	Cp17	9.85bc	22.74ab	32.7ab
	Cp41	10.93ab	20.04cde	31bcde
	Control	10.88ab	21.04abcd	32bc
LSD (0.05)		**1.08**	**2.25**	**1.99**
CV (%)		**8.17**	**7.77**	**4.7**

Mean values within column designated with similar letters are not significant each other at $p<0.05$, LSD (5%) = Least significant difference at $p<0.05$ and CV (%) = percentage of coefficient of variation.

Effect of variety and *Rhizobium* strain on seedling vigour index

An analysis of variance revealed that the *Rhizobium* strain and chickpea variety had a substantial impact on both vigor index I and vigor index II. While variety and *Rhizobium* strain interaction also affects vigor index I, it has no discernible effect on vigor index II. Farmers' cultivar had the lowest vigor index II (56.26) while the Dimtu variety had the highest vigor index II (62.85). The Cp17 strain had the highest vigor index II (60.73) and was statistically not significant with the Cp11 and Cp41 strains. On the other hand, the non-inoculation strain had the lowest vigor index II (57.93) (Table 5).

Table 5. Main effects of variety and *Rhizobium* strain on vigor index II of the chickpea variety

Variety	VI2
Eshete	59.08b
Dimtu	62.85a
Teketay	59.98b
Farmers cultivar	56.26c
LSD (0.05)	**1.79**
R Strains	
Cp 11	59.62ab
CP 17	60.73a
Cp 41	59.9a
Control	57.93b
LSD (0.05)	**1.79**
CV (%)	**4.23**

Mean values within columns designated with similar letters are not significant to each other at p<0.05, LSD (5%) = Least significant difference at p<0.05 and CV (%) = percentage of coefficient of variation.

Interaction effect of variety and *Rhizobium* strain on vigour index one

Both the variety and the *Rhizobium* strain inoculation, as well as the interaction between the two parameters, had a substantial impact on the chickpea vigor index I. The cultivar Farmers inoculated by the Cp11 strain had the highest vigor index I (3340), which was statistically equal to the vigor index I for the cultivar Farmers inoculated by the Cp17 strains and no inoculation, as well as the Teketay variety inoculated by Chp11. Table 6 shows the lowest vigor index I for the Dimtu variety without inoculation (2204).

Table 6. Interaction effects of Variety and *Rhizobium* strain on vigor index I of chickpea varieties

Variety	R strain	Vigor index I
Eshete	Cp11	2835.8fg
	Cp17	3078.3bcde
	Cp41	2994.5cdef
	Control	2867efg
Dimtu	Cp11	2568.8h
	Cp17	2666.3gh
	Cp41	2827fg
	Control	2204i
Teketay	Cp11	3201abc
	Cp17	2846.3fg
	Cp41	2946def
	Control	2880.3ef
Farmers cultivar	Cp11	3340a
	Cp17	3219.8ab
	Cp41	3035.3bcdef
	Control	3152.5abcd
LSD (0.05)		**211.7**
CV (%)		**5.09**

Mean values within column designated with similar letters are not significant each other at p<0.05, LSD (5%) = Least significant difference at p<0.05 and CV (%) = percentage of coefficient of variation.

Discussion

The chickpea varieties exhibited inherent variability for the examined seed physiology parameters, as evidenced by the considerable disparity in crop germination speed reported among them. For the Farmers cultivar, more seedlings emerged each day. Additionally, the Eshete type took longer to emerge each day than the Dimtu & Teketay species. Fast germination is a crucial aspect of seed vigor, according to Toumey & Korstian (1942), and it typically translates into faster seedling emergence in the field. High-germination-speed seeds were shown to be vigorous in the field and able to withstand adverse environments. According to the study, there are notable differences between chickpea types and the inoculation of *Rhizobium* strains for a typical percentage of chickpea seedlings. This suggested that the fundamental properties of the normal seedling percentage varied significantly across the chickpea types, potentially due to genetic factors and strain compatibility with *Rhizobium*. When inoculated with all *Rhizobium* strains, the Farmers cultivar enhanced normal seedlings, while the Teketay variety was more receptive to the Cp11 and Cp17 strains. When inoculated with the Cp17 strain and when not inoculated, the Dimtu and Eshete cultivars enhanced the usual seedling percentage. According to Adebisi (2011), seed size is one of the factors that determines the quality of the seed and has an impact on the germination and overall performance of the plant. The study found that strains of *Rhizobium* and varieties of chickpeas have a major impact on the dry weight of seedlings. This suggested that both varietal diversity and the compatibility of the *Rhizobium* inoculation affected the dry weight of seedlings. The chickpea variety that was inoculated with Cp17 and Cp41 resulted in an increase in weight, and the dimtu variety was assessed higher than the other kinds. According to Moshatati & Gharineh (2012), greater food stores in the seeds may account for the higher seedling weight of the heavy seeds. The length of the chickpea shoots and roots were significantly affected by the inoculation of *Rhizobium* strains and kinds, according to the results. Farmers cultivar variety enhanced shoot length when inoculated with CP11, 41 *Rhizobium* strains and no inoculation. Eshete variety was more susceptible to the Cp17, Cp41 *Rhizobium* strains and increased the shoot of plants. Farmer's cultivar was more responsive to CP11, 17 *Rhizobium* strains, and no inoculation, and it also expanded the root of the plants. Teketay variety was most receptive to three *Rhizobium* strains and no inoculation. According to Moshatati & Gharineh (2012), significant food reserves in seeds may be the cause of longer seedlings. The study found that there were inherent changes in the examined seed quality characteristics between the *Rhizobium* strain and the chickpea varieties, as evidenced by the significant differences identified in the seedling vigor index of the crop. Samson (2013) found that varietal variation in two teff cultivars had an impact on the seed vigour index. The symbiotic interaction between *Rhizobium* and legumes is quite specialized, with most legume plants forming associations with a restricted number of *Rhizobium* strains. The results of the study showed that vigour index one of chickpea was significantly affected by the inoculation of *Rhizobium* strains and types. The Eshete variety had a higher vigor index in the crop and showed greater responsiveness to the two *Rhizobium* strains (Cp17 and Cp41). When the Farmers cultivar was inoculated with Cp11 and Cp41 strains and non-inoculation, vigor index I rose. A seed's vigor index is the total of its constituent traits that establish its potential activity level and ability to survive a variety of field conditions. According to Zewdie (2004), seedlings with a well-developed shoot and root system are more resilient to unfavorable field conditions and offer improved seedling emergence and establishment. As a result, seedlings with a higher index are anticipated to germinate and emerge quickly, ultimately enabling them to escape unfavorable field conditions.

Conclusion

Africa's leading producer of chickpeas is Ethiopia. The 1.9 tons ha^{-1} average productivity of Ethiopian chickpeas is less than half of the 5.5 tons ha-1 potential output. Rhizobial strains were used to increase chickpea seed output and seed quality. High-quality seeds should germinate well and sprout quickly, as well as thrive vigorously. For farms to operate at their best and provide the highest yields, seed quality is crucial. *Rhizobium* inoculation of the seed is a practical method of attaining sustainable output and may replace expensive N-fertilizers. Thus, the purpose of this study was to evaluate how various *Rhizobium* strains affected the seed quality of four distinct types of chickpeas. Overall, the experiment results demonstrated that *Rhizobium* strain and the combination of the two factors had a substantial impact on seed quality measures after the chickpea seeds of varying quality were grown. High-quality seeds were often produced by the enhanced variety' seeds that were inoculated with the Cp17 strain. Therefore, it is advised to take into account strains of *Rhizobium* and varieties in order to generate high-quality seeds and increase chickpea productivity.

Acknowledgement

I would like to express my deepest gratitude to Prof. Wassu Mohammed and Ambo Agricultural Research Center members for their encouragement from the start to the completion of my study.

Author contributions

Berhanu Soboka wrote the proposal, conducted the research, data collection and wrote the manuscript. Mr. Chaluma Tujuba, reviewed, analyzed and edited the manuscript, formatted the manuscript according to the journal guidelines.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

No AI tools have been used in manuscript preparation.

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