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Seed Germination and Seed Vigour Induction through Foliar Application of Plant Growth Regulators and Nutrients under Drought Stress in Chickpea (*Cicer arietinum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea cultivation in rainfed regions, such as Madhya Pradesh, faces challenges due to water stress, impacting crop yield and quality. The study conducted assesses the impact of foliar spray with plant growth regulators and nutrients on the germination percentage, seedling length, and seed

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vigour indices of chickpea varieties (JG 36 and JG 14) under water deficit conditions. Cytokinin analogues, known for breaking dormancy, and other growth regulators and nutrients were applied during the flowering stage. The results revealed significant differences in germination percentage, seedling length, seed vigour index I, and seed vigour index II among irrigation levels, varieties, and foliar spray of plant growth regulators and nutrients. Under different irrigation levels, D_1 (Irrigation at 30 DAS and at flower initiation) recorded the highest germination percentage (97.24%), seedling length (31.41 cm), seed vigour index I (3054.81), and seed vigour index II (52.77) compared to control D_2 (Drought stress at Flowering up to physiological maturity). JG 14 (V1) performed well, achieving the highest germination percentage of 96.99%. Among the treatments, foliar application of Benzyladenine (BA) at 40 ppm (T_3) resulted in the highest germination percentage (98.17%) and seed vigour index II (60.33), whereas T_8 (BA 40ppm + $ZnSO_4$ 1%) exhibited the highest seedling length (31.83 cm) and seed vigour index I (3099.85). This study demonstrates that foliar spray with plant growth regulators and nutrients, particularly Benzyladenine at 40 ppm, significantly improved germination percentage, seedling length, and seed vigour index, offering potential solutions for enhancing chickpea productivity under water deficit conditions.

Keywords: Seed quality; benzyladenine; water deficit stress; thidiazuron; plant growth regulators.

1. INTRODUCTION

Globally, Chickpea is prevalent in regions with temperate and subtropical climates [1]. In Madhya Pradesh, chickpea is often grown as a rainfed crop, utilizing the monsoon rainfall during the Rabi season [2]. The crop's resilience to withstand water stress and its ability to perform nitrogen fixation are crucial in rainfed agriculture, particularly in regions with limited irrigation facilities [3]. In India, drought is one of the predominant constraints on production within semi-arid ecosystems. Salinity, high temperatures, and low temperatures, water stress also negatively impact plant growth and agricultural productivity in these regions [4]. Among the various abiotic stresses, drought is identified as the most severe, significantly restricting crop production and yield, especially in arid and semi-arid areas [5]. Severe water deficit condition leads to reduction in crop growth, physiology and yield of the crop [6].

Drought stress results in a decrease in both germination percentage and seedling growth [7]. In the grain-filling stage of the crop, a water deficit becomes a crucial factor, restricting the rate of seed filling and various processes associated with seed quality. The productivity of chickpea, particularly in semi-arid regions, is connected to factors such as insufficient photosynthates in pods and seed setting. The application of macro and micro nutrients through foliar means has been suggested as a better solution [8]. Water stress is identified as the primary abiotic stress that hampers crop growth, development, and overall production on a globally [9].

The application of bioregulators from external sources has emerged as an innovative technology for enhancing stress tolerance in crop plants [10]. The importance of combination of bio-regulators and nutrients in enhancing crop yields has long been recognized, and this cost-effective technology has now demonstrated its value in substantially increasing agricultural productivity. The challenge of moisture stress during the flowering and seed-setting stages, leads to decline in seed yield [11]. Thiourea is recognized for its ability to break dormancy and promote germination [12]. In potato tuber, application of thiourea at optimal concentration enhances the germination process and the development of multiple sprouts [13].

Thiourea, also recognized as thiocarbamide due to its nitrogen ($-NH_2$) and sulfur (-SH) elements, plays a role in influencing plant growth under stressful conditions [14]. Additionally, it has been observed to facilitate seed germination, promote growth, and alleviate stress in plants [15]. The effectiveness of thiourea extends to overcoming both environmentally induced and inherent seed dormancy by fostering seed germination [16]. Thiourea increased seed germination, foliar application led to enhanced gas exchange properties, and supplementing the medium supported improved root growth and proliferation [15].

According to Kulsumbi et al. [17], the application of Thiourea at a 1% concentration resulted significantly superior seed quality parameters compared to the control, including speed of germination, shoot length, root length, seedling dry weight, and seedling vigour index. Yasar et al. [18] found that germination percent was more on 50 µM BAP than 10 µM Thidiazuron (TDZ) in pea seeds. TDZ leads to a reduction in the percentage of seed germination [19]. This inhibitory effect of higher TDZ levels on seed germination has been documented in various plant species, including instances in *Carica papaya* and *Lotus corniculatus* [20-22]. Hence, this study was taken up to evaluate the efficiency of foliar application of macro and micro nutrients on seedling quality parameters of chickpea.

2. MATERIALS AND METHODS

The chickpea varieties JG 36 (V1) and JG 14 (V2) were sown at the Experimental Research Farm, Seed Technology Research Unit, JNKVV, Jabalpur, during the Rabi seasons of 2021-2022 and 2022-2023 in a split-split plot design with three replications. The experimental area is located at a latitude of 23°12' N and a longitude of 79°56' E, situated at an elevation of approximately 390 meters above mean sea level. According to U.S. classification, the soil type of the experimental area is categorized as 'Vertisol'. Using soil moisture content data and soil water potential data, the water deficit conditions of the crop were assessed. During the water deficit condition, the foliar spray of plant growth regulators and nutrients was applied at the 50% flowering stage for both D_1 - Control (Irrigation at 30 DAS and at flower initiation) and D_2 - Drought (Drought stress at Flowering upto physiological maturity). During flowering stage, irrigation was provided only to D_1. Water stress was imposed in D_2 during the reproductive stage.

In both the conditions, different plant growth regulators and nutrients were applied at 50% flowering stage *viz*., T_1 - Control (no spray), T_2- Thiourea (TU) @ 1000 ppm, T_3 – Benzyladenine (BA) @ 40 ppm, T_4 – Thidiazuron (TDZ) @ 10 ppm, T_5 – 1% $ZnSO_4$, T_6 – 1% KCl, T_7 – TU @ 1000 ppm + 1% $ZnSO_4$, T_8 - BA 40 @ ppm + 1% $ZnSO_4$, T_9 - TDZ @ 10 ppm + 1% $ZnSO_4$, T_{10} - TU @ 1000 ppm + 1% KCl, T_{11}- BA @ 40 ppm+ 1% KCl, T12- TDZ @ 10 ppm + 1% KCl. After the harvest, the seeds were subjected to a germination test following the International Seed Testing Association (ISTA) procedure, which involved the paper towel method in a germinator.

2.1 Procedure

Three replications of 100 seeds each from their respective treatments were taken. The paper towel was moistened with distilled water, ensuring it was damp but not dripping wet. The moistened paper towel was placed on a flat surface. On one half of the paper towel, seeds were aligned evenly, maintaining consistent spacing between them. The other half of the moistened paper towel was folded over the seeds to fully cover them. An additional layer of butter paper was used to fold the paper towel, creating a humid microenvironment conducive to germination (Fig. 1). To further support this environment, the paper towel was kept in a tray filled with water. The samples were then placed in a germinator at a temperature of 25 ± 2°C for 10 days, maintaining a relative humidity of 90% [23]. The tray's water level was refilled as needed. After a 10-day period, the germinated seeds were categorized as normal seedlings, abnormal seedlings, hard seeds, or dead seeds. The germination percentage was then calculated exclusively based on the count of normal seedlings.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Fig. 1. Seed quality testing of chickpea seeds using the paper towel method

2.1.1 Seedling length (cm)

Five normal seedlings from the germination test were randomly selected for the measurement of seedling length on the tenth day [24]. The seedling length was measured from the collar region to the tip of the primary leaf. The mean seedling length of 10 seedlings was expressed in centimeters.

2.1.2 Seed vigour index I

Seed vigour Index is a measure used to assess the overall vigour or health of seedlings. Seed vigour Index (I) was calculated using the formula given by Abdul-Baki and Anderson [25].

$$\text{Vigour Index I} = \text{Seedling length (cm) x Germination percentage}$$

2.1.3 Seed vigour index II

The weight of 10 seedlings (grams), excluding the cotyledons, was measured on the 10th day after drying them at 60-80°C in an oven for 24 hours. The lot of seedlings that displayed the highest dry weight was considered to be the most vigorous. This method helps assess the seedlings' overall health and growth potential based on their accumulated dry weight, excluding the energy reserves stored in the cotyledons. Seed vigour Index (II) was calculated using the formula given by Abdul-Baki and Anderson [25].

$$\text{Vigour index II} = \text{Seeding dry weight (g) x Germination percentage}$$

2.2 Statistical Analysis

The statistical analysis, namely two-way ANOVA, and Tukey's Honest Significant Difference (HSD) test at a 5% level of significance, was conducted using R 4.2.2 statistical software. All results were expressed as the average of three replications. Treatment effects were determined through analysis of variance using the Split-Split plot design [26].

3. RESULTS AND DISCUSSION

The analysis of variance conducted on data of two consecutive years (2021-2022 and 2022-2023), as well as the pooled data revealed a significant variation in germination percentage, seedling length, seed vigour index I, and seed vigour index II (Table 2).

3.1 Effect of Foliar Spray of Plant Growth Regulators and Nutrients on Germination Percentage and Seedling Length (cm) of Chickpea Seeds under Water Deficit Stress

Seed quality is influenced by various factors including environmental conditions, genetic factors, and the moisture and fertility of the soil [27]. Seed germination stands out as an important and highly sensitive stage in the plant life cycle [28]. Seed germination and seedling growth significantly contributes for determining the yield of the crop [29]. The results from the pooled analysis of two consecutive years (Table 1 and Fig. 3) indicates the range of germination percentage was found to be 95.58% to 98.17%. Under the irrigation level, germination percentage was found to be significant. The highest (97.24%) germination percentage was found in D_1 (Irrigation at 30 DAS and at flower initiation) followed by 96.33% in D_2 (Drought stress at Flowering upto physiological maturity). Among the varieties, V_1 (JG36) recorded highest (96.99%) germination percentage followed by 96.58% in V_2 (JG 14). Among the treatments, it was observed that foliar application of Benzyladenine (BA) at 40 ppm (T_3) resulted in the highest germination percentage (98.17%) which is at par with all other treatments. In contrast, the control treatment (T_1) recorded the lowest (95.58%) germination percentage which is on par with treatment T_2 (96.21%).

Our result is in similarity with Morsy et al., [30] who stated that seed germination (%) decreased with increase in water deficit condition. Seeds produced under moisture deficit conditions exhibited delayed and uneven germination, along with reduced seedling growth and storability [31]. Water deficit stress during the vegetative stage has adverse effects on both seed germination and plant establishment. However, legume plants exhibit drought tolerance during the late vegetative stage of the crop [32]. Okcu et al. [33] found that the primary effect of drought is impaired germination and early seedling growth, ultimately impacting the growth and productivity of legume [34].

Swain et al. [35] have noted that early stage of the crop particularly seed germination and initial seedling growth, are critical stages that are adversely affected by water stress. The results from the pooled analysis of two consecutive years (Table 1) indicate the range of seedling length was found to be 26 cm to 31.83 cm. With respect to irrigation levels, seedling length was found to highest (31.41 cm) in D1 (Irrigation at 30 DAS and at flower initiation). Among the varieties, highest (30.30 cm) seedling length was found to be observed in V_2 (JG 14) followed by V_1 (JG 36) with 29.6 cm (Fig. 2). Among the treatments, maximum (31.83 cm) seedling length was observed for treatment T_8 - BA 40 @ ppm + 1% $ZnSO_4$ which is on par with all other treatments, whereas minimum seedling length was observed for control T_1 (26.00 cm) which is on par with T_2 (28.64 cm), T_6 (29.77 cm), T_7 (28.29 cm), T_9 (28.98 cm) and T_{10} (30.21 cm) respectively. Our result is in similarity with Soleymani et al. [27] who reported that there was a significant reduction in germination percentage, seedling growth as the stress level increases. Mohammadkhani and Heidari [36] also observed

Fig. 2. Seedling length affected by foliar spray of BA 40 @ ppm + 1% $ZnSO_4$ in the JG 14 chickpea variety under water deficit stress condition

Table 1. Germination percentage and seedling length as affected by varied irrigation level, varieties, foliar spray of plant growth regulators and nutrients in chickpea

Treatments	**Germination percentage**			**Seedling length (cm)**		
	Main plot: Irrigation (D)					
	2021-2022	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**
D_1	97.39^a	97.10^a	97.24^a	31.80^a	31.02^a	31.41^a
D_2	96.36^b	96.29^a	96.33^b	28.41^b	28.57^b	28.49^b
SEm±	0.10	0.16	0.12	0.07	0.10	0.06
SD	0.14	0.22	0.17	0.14	0.09	0.09
Subplot: Varieties (V)						
V_1	97.21^a	96.76^a	96.99^a	30.17^a	29.04^b	29.60^b
V_2	96.54^b	96.62^a	96.58^b	30.05^a	30.55^a	30.30^a
SEm±	0.06	0.08	0.03	0.05	0.11	0.04
SD	0.08	0.11	0.04	0.15	0.07	0.06
Sub-sub plot: Treatments (T)						
T_1	95.83^d	95.33^e	95.58^f	26.50^d	25.51^c	26.00^b
T_2	96.25^{cd}	96.17^{de}	96.21^{ef}	30.44^{abcd}	26.83^{bc}	28.64^{ab}
T_3	98.25^a	98.08^a	98.17^a	34.53^a	28.43^{abc}	31.48^a
T_4	97.25^b	97.33^{abc}	97.29^{bc}	32.84^{ab}	29.71^{abc}	31.28^a
T_5	96.75^{bc}	96.50^{bcd}	96.63^{bcde}	31.78^{ab}	31.40^a	31.59^a
T_6	96.50^{bcd}	96.42^{cde}	96.46^{de}	29.44^{bcd}	30.09^{ab}	29.77^{ab}
T_7	96.50^{bcd}	96.50^{bcd}	96.50^b	27.13^{cd}	29.46^{abc}	28.29^{ab}
T_8	97.08^{bc}	97.58^{ab}	97.33^{bcde}	$31.11a^{bc}$	32.56^a	31.83^a
T_9	96.83^{bc}	96.92^{bcd}	96.88^{cde}	28.74^{bcd}	29.22^{abc}	28.98^{ab}
T_{10}	96.83^{bc}	96.33^{cde}	96.58^{bcd}	29.06^{bcd}	31.37^{ab}	30.21^{ab}
T_{11}	97.17^b	96.92^{bcd}	97.04^{bcde}	28.94^{bcd}	32.33^a	30.64^a
T_{12}	97.25^b	96.25^{cde}	96.75^{bcde}	30.78^{abcd}	30.62^{ab}	30.70^a
SEm±	0.19	0.23	0.15	0.95	0.96	0.93
SD	0.26	0.33	0.22	1.36	1.31	1.31

The values with same letter cases are not significantly different at p<0.05 level

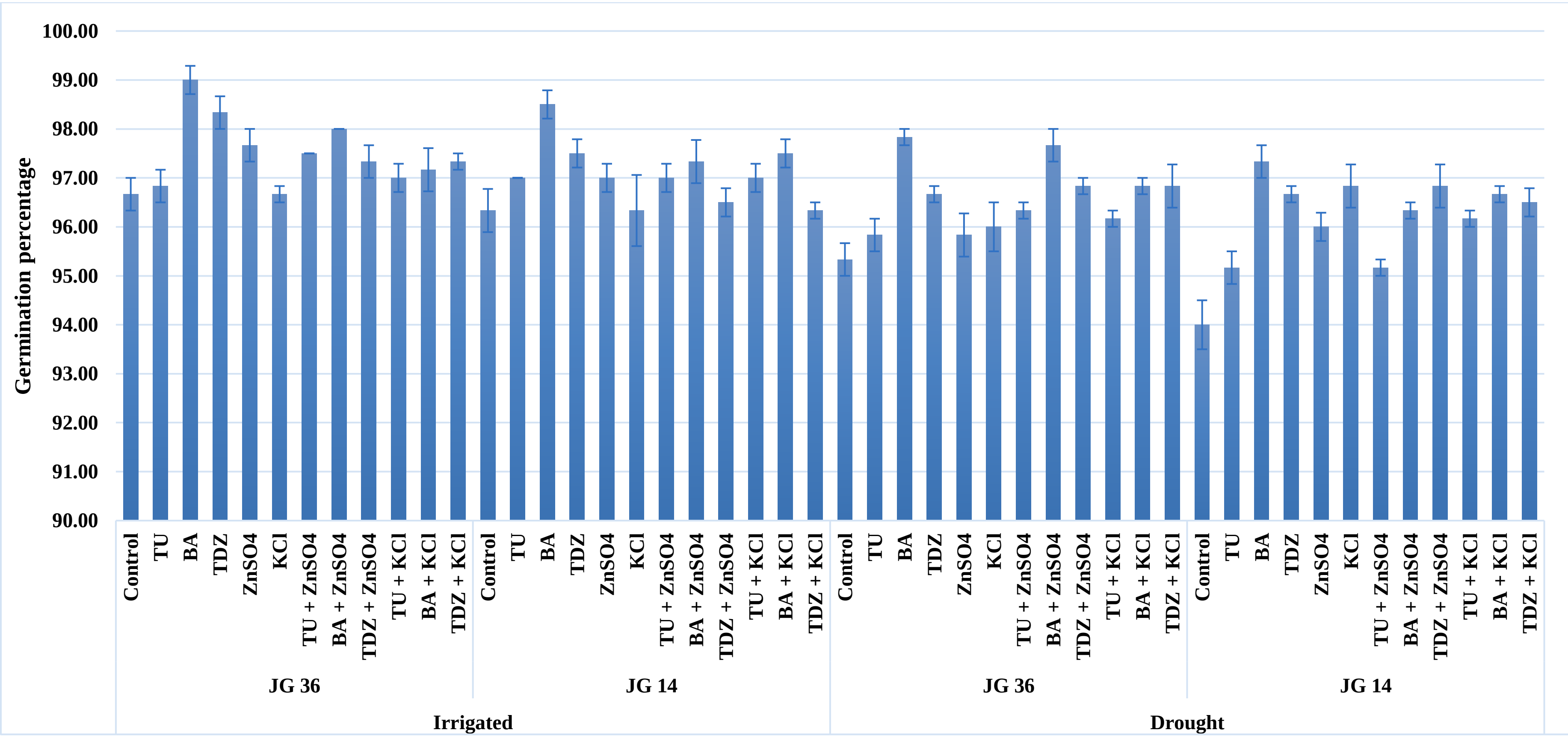

Fig. 3. Effect of foliar spray of plant growth regulators and nutrients on the seed germination percentage of chickpea varieties under water deficit stress

TU – Thiourea @ 1000 ppm, BA – Benzyladenine @ 40 ppm, TDZ - Thidiazuron @ 10 ppm, $ZnSO_4$ - 1% ZnSO4, KCl - 1% KCl

Table 2. Results of the two-way ANOVA and Tukey multiple range tests for the comparative effects of plant growth regulators and nutrients, irrigation levels and varieties on the seed germination percentage, seedling length (cm), seed vigour index I and Seed vigour index II of Chickpea under water deficit stress

Treatments	Germination percentage			Seedling length (cm)			Seed Vigour Index I			Seed Vigour Index II		
	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
D	0.61*[a]	0.96ns	0.73*	0.39***	0.62**	0.37***	43.65***	41.17**	32.58***	1.31**	0.46**	0.89**
V	0.23**	0.32ns	0.13***	0.20ns	0.48***	0.16***	13.52**	35.05***	13.31***	0.63***	0.40**	0.35***
T	0.53***	0.66***	0.44***	2.60***	2.69***	2.60***	252.41***	263.09***	253.36***	4.46***	4.55***	4.46***
D x V	0.45ns	0.70ns	0.49ns	0.32*	0.58*	0.29ns	31.24**	43.98*	24.88ns	1.06ns	0.50***	0.67***
D x T	0.85*	1.05**	0.79***	3.49ns	3.62*	3.50ns	337.65ns	351.83*	338.66ns	5.99***	6.09ns	6.00ns
D x V x T	1.09**	1.37ns	0.95ns	4.92***	5.11ns	4.94ns	477.19***	497.82ns	478.81ns	8.46***	8.61***	8.45*

*[a] F-values. ns: not significant F ratio (p < 0.05); *, ** and ** indicate significance at P < 0.05, 0.01 and 0.001, respectively*

a reduction in root and shoot length, as well as the fresh and dry weight of maize seedlings with an increase in stress levels.

3.2 Effect of Foliar Spray of Plant Growth Regulators and Nutrients on Seed Vigour Index I and Seed Vigour Index II of Chickpea Seeds under Water Deficit Stress

The results from the pooled analysis of two consecutive years (Table 3) indicate the range of seed vigour index I was found to be 2487.11 to 3099.85. Seed vigour index I was found to be highest (3054.81) in D_1 (Irrigation at 30 DAS and at flower initiation) followed by D_2 (2745.37) (Drought stress at Flowering upto physiological maturity). With respect to varieties, seed vigour index I was found be higher (2927.7) in V_2 (JG 14) followed by 2872.48 in V_1 (JG 36). With respect to the foliar application of plant growth regulators and nutrients, highest (3099.85) seed vigour index I was found to be in treatment T_8 (BA 40 @ ppm + 1% $ZnSO_4$) which is in on par with all other treatments except T_1. Seed vigour index I was found to be lowest (2487.11) in control T_1 which is on par with T_2 (2756.39), T_6 (271.78), T_7 (2731.74) and T_9 (2807.85), respectively. According to Dhandas et al. [37], the traits most susceptible to drought stress are seed vigour index and plumule length.

The results from the pooled analysis of two consecutive years (Table 3) indicate the range of seed vigour index II was found to be 46.81 to 60.33. Under the irrigation level, seed vigour index II was found to be significant. The highest (52.77) seed vigour index II was found in D_1 (Irrigation at 30 DAS and at flower initiation) followed by 49.91 in D_2 (Drought stress at Flowering upto physiological maturity). Among the varieties, V_1 (JG36) recorded highest (53.13) seed vigour index II followed by 49.57 in V_2 (JG 14). Among the treatments, it was observed that foliar application of Benzyladenine @ 40 ppm (T_3) resulted in the highest seed vigour index II (60.33) which is on par with T_4 (53.16). In contrast, the control treatment (T_1) recorded the lowest seed vigour index II 46.81 which is at par with treatment T_3 (60.33). Our result is in

Table 3. Seed vigour Index I and Seed vigour Index II as affected by varied irrigation level, varieties, plant growth regulators and nutrients of chickpea

Main plot: Irrigation (D)						
	Seed Vigour Index I			Seed Vigour Index II		
Treatments	**2021-2022**	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**
D_1	3097.90^{a}	3011.72^{a}	3054.81^{a}	52.79^{a}	52.74^{a}	52.77^{a}
D_2	2738.36^{b}	2752.39^{b}	2745.37^{b}	48.53^{b}	51.32^{b}	49.91^{b}
SEm±	7.17	6.77	5.35	0.22	0.08	0.15
SD	10.14	9.57	7.57	0.30	0.11	0.21
Subplot: Varieties (V)						
V_1	2934.09^{a}	2810.86^{b}	2872.48^{b}	53.84^{a}	52.41^{a}	53.13^{a}
V_2	2902.16^{b}	2953.25^{a}	2927.70^{a}	47.48^{b}	51.65^{b}	49.57^{b}
SEm±	3.44	8.93	3.39	0.16	0.10	0.09
SD	4.87	12.62	4.79	0.23	0.15	0.12
Sub-sub plot: Treatments (T)						
T_1	2538.33^{d}	2435.89^{c}	2487.11^{b}	44.03^{d}	49.58^{b}	46.81^{b}
T_2	2929.18^{bcd}	2583.60^{bc}	2756.39^{ab}	49.97^{bcd}	51.93^{ab}	50.95^{b}
T_3	3392.71^{a}	2788.13^{abc}	3090.42^{a}	65.66^{a}	54.99^{ab}	60.33^{a}
T_4	3194.68^{ab}	2890.88^{ab}	3042.78^{a}	53.47^{bc}	52.85^{ab}	53.16^{ab}
T_5	3074.93^{ab}	3028.25^{a}	3051.59^{a}	45.49^{d}	59.00^{a}	52.24^{b}
T_6	2841.38^{bcd}	2902.18^{ab}	2871.78^{ab}	45.02^{d}	51.29^{b}	48.15^{b}
T_7	2618.86^{cd}	2844.63^{abc}	2731.74^{ab}	46.27^{cd}	49.89^{b}	48.08^{b}
T_8	3022.24^{abc}	3177.45^{a}	3099.85^{a}	54.09^{b}	49.56^{b}	51.83^{b}
T_9	2783.28^{bcd}	2832.43^{abc}	2807.85^{ab}	48.58^{bcd}	54.64^{ab}	51.61^{b}
T_{10}	2814.95^{bcd}	3021.20^{ab}	2918.08^{a}	49.65^{bcd}	52.50^{ab}	51.07^{b}
T_{11}	2812.63^{bcd}	3133.39^{a}	2973.01^{a}	51.49^{bcd}	47.51^{b}	49.50^{b}
T_{12}	2994.39^{abc}	2946.62^{ab}	2970.50^{a}	54.23^{b}	50.62^{b}	52.43^{b}
SEm±	89.81	93.61	90.15	1.59	1.62	1.59
SD	127.01	132.39	127.49	2.25	2.29	2.24

The values with same letter cases are not significantly different at $p<0.05$ level conformity with Abdoli et al. (2012) found that drought stress led to a decrease in seedling vigour index. Movahhedy-Dehnavy et al. [38] reported that the foliar application of nutrients increased the germination percentage, seedling dry weight, and seed vigour index [39]. Uniform germination rate and seedling emergence are important factors for establishing a good crop stand [40].

4. CONCLUSION

In rainfed chickpea cultivation, water stress poses challenges to yield and seed quality. The germination percentage, seedling length, and seed vigour index were significantly affected by water deficit stress condition. Our findings underscore the importance of foliar application of plant growth regulators and nutrients, particularly the application of Benzyladenine at 40 ppm, in mitigating the adverse effects of water deficit conditions on seed quality parameters. The seed vigour index I and seed vigour indexII reflected the positive impact of foliar applications, with specific treatments, such as T_8 (BA 40 ppm + $ZnSO_4$ 1%), outperforming others. These results provide practical insights for optimizing chickpea yield and seedling performance under water deficit stress, offering a promising avenue for sustainable crop management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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