

# Phenological Variation in Chickpea (*Cicer arietinum* L.) varieties through Foliar Application of Cytokinin Analogs and Nutrients Under Water Deficit Stress

## Abstract

Chickpea (*Cicer arietinum* L.), a rainfed crop predominantly grown in temperate and subtropical climates, faces significant challenges in production due to terminal drought stress impacting various phenological stages. This study addresses the challenges posed by terminal drought stress on the phenological stage of chickpea varieties viz., JG 36 and JG 14. The experiment was conducted during the Rabi seasons of 2021-2022 and 2022-2023 at the Experimental Research Farm, Seed Technology Research Unit, JNKVV, Jabalpur (M.P), using a split-split plot design with three replications. This research also investigates the impact of foliar applications of cytokinin analogs viz., Thiourea, Thidiazuron, and Benzyladenine and nutrients viz., ZnSO<sub>4</sub> and KCl on chickpea under water deficit stress conditions. The results revealed significant differences in days to pod formation, seed formation, physiological maturity, and harvest maturity among the irrigation levels, varieties, and foliar spray of plant growth regulators and nutrients. Under different irrigation levels, D<sub>1</sub> (Irrigation at 30 DAS and flower initiation) exhibited delayed phenological stages of the crop, while D<sub>2</sub> (Drought stress at flowering up to physiological maturity) showed an early onset of all the phenological stages. Under water deficit conditions, JG 14 exhibited accelerated maturity beyond its typical early maturation in comparison to well-watered conditions, highlighting the impact of environmental stress on varietal responses. With respect to the application of plant growth regulators and nutrients, treatment T<sub>12</sub> (TDZ @ 10 ppm + 1% KCl) significantly delayed pod and seed formation, as well as physiological and harvest maturity as compared to untreated control (T<sub>1</sub>). Foliar application of TDZ @ 10 ppm + 1% KCl (T<sub>12</sub>) enhanced seed filling duration by 2.59 days compared to the untreated control. Further investigations are needed to identify the impact of Thidiazuron and KCl in enhancing seed yield and seed weight of chickpeas under optimal and sub-optimal soil water conditions, to provide recommendations for chickpea growers.

**Keywords:** Benzyladenine; Chickpea; Phenology; Thidiazuron; Water deficit stress.

## 1. Introduction

Chickpea (*Cicer arietinum* L.), belonging to the Leguminosae family, is commonly known by Bengal gram, garbanzo beans, and Egyptian peas. Chickpea is prevalent in regions with temperate and subtropical climates (FAOSTAT, 2011). The primary limitation of chickpea cultivation is predominantly attributed to terminal drought which is due to the traditional cultivation practice of growing chickpea as a winter crop, relying on either conserved soil moisture or restricted irrigation facilities (Tiwari *et al.*, 2020). Worldwide chickpea production and productivity are affected by the increase in drought, high temperature, low temperature, and excessive moisture, affecting the crop yield (Devasirvatham *et al.*, 2018). In Chickpea, yield loss may be due to intermittent drought during the vegetative phase, drought stress during reproductive development, or terminal drought at the end of the crop cycle (Serraj *et al.*, 2004).

Drought is one of the major constraints for the growth and development of legumes affecting their ability to produce flowers and flowering period, seed germination resulting in reduced pod and grain yield (Chowdhury *et al.*, 2016; Pushpavalli *et al.*, 2015). Drought stress has an impact on crop productivity by negatively influencing plant growth, physiology, nutrient and water relations, photosynthesis, and assimilate partitioning (Barnaba *et al.*, 2008; Praba *et al.*, 2009). Under rainfed conditions, the crop frequently faces the water deficit stress condition especially during the reproductive stage of the crop (Turner *et al.*, 2004), accounting for 40% - 45% of global chickpea yield losses (Ahmed *et al.*, 2005). During the grain-filling stage of the crop, the water deficit condition reduces the rate of seed filling and seed quality (Keerthana *et al.*, 2024).

Phenology plays a vital role in the adaptation of crops to various environments, and its variation depends on factors such as genotype, soil moisture, sowing time, and also geographical location (Sachdeva *et al.*, 2022). Plants can mitigate the impact of terminal drought by exhibiting early phenology (Devasirvatham *et al.*, 2018). Climate change adversely affects soybean production and productivity, as erratic weather events such as drought, high temperature, *etc.*, disrupt the plant's phenology, metabolism, and various physiological processes (Keerthana *et al.*, 2022). Berger *et al.*, (2003) state that phenological development is a major factor in the adaptation of chickpeas to environments with limited water availability.

PGRs play an important role in regulating various physiological processes in plants, allowing them to cope with and adapt to environmental stresses, including drought, salinity, and other adverse conditions. Among plant growth regulators, cytokinins (CKs) are known to regulate several aspects of plant growth and development, including the response of plants to abiotic stress (Rivero *et al.*, 2007). Cytokinins can regulate various physiological processes in plants and also provide protective effects under stress conditions (Vineeth *et al.*, 2016).

Thiourea (TU), a sulfhydryl compound chemically referred to as thiocarbamide, external application of TU has been found to promote the growth and productivity of plants, both in favourable environmental conditions and under stress conditions (Patade *et al.*, 2020). Benzyladenine (BA) stimulates cell division in plants and tends to decline when plants are exposed to environmental stress (Hu *et al.*, 2020). The specific mechanism of action of Thidiazuron (TDZ) remains unclear, but studies suggest its potential to impact cytokinin biosynthesis and metabolism (Mok *et al.*, 2000). Additionally, TDZ has been reported to enhance fruit growth and development, delay senescence, improve stress tolerance, and increase overall crop yield (Nisler, 2018). TDZ treatment has been shown to induce gene expression related to ethylene biosynthesis and signaling, leading to earlier ethylene accumulation in leaves compared to the abscission zone (Li *et al.*, 2022). Thidiazuron (TDZ) was observed to extend the time to petal blackening by approximately two days, although it did not lead to bud opening (Imsabai *et al.*, 2013). And also in tulips, cytokinins were found to delay flower senescence (Van Doorn *et al.*, 2011).

Under water deficit conditions, plants face challenges in absorbing an adequate amount of nutrients from the soil, leading to nutrient deficiency. So, the external application of essential nutrients alleviates nutrient deficiency and enhances water stress tolerance in crops (Hussain *et al.*, 2020). Zinc plays a crucial role in the reproductive development of plants, influencing flower initiation, floral development, as well as male and female gametogenesis, fertilization, and seed development (Pathak *et al.*, 2012). Zinc deficiency also results in delayed crop maturity, associated with reduced water use and decreased water use efficiency (Khan *et al.*, 2003). Potassium (K), an essential nutrient and an abundant cation found in plants, has a major role in plant physiological processes such as photosynthesis, assimilate

transportation, and activation of enzymes (Hasanuzzaman *et al.*, 2018). The application of potassium at the vegetative stage of the pulse crop boosts the crop growth rate, yield, and yield components under water deficit conditions (Thalooth *et al.*, 2006).

No work has been done on utilizing the combination of cytokinin analogs, *viz.*, Thiourea, Thidiazuron, and Benzyladenine, along with nutrients, as foliar applications to alleviate drought stress in chickpea. Therefore, our research aimed to test three different cytokinin analogs *viz.*, Thiourea, Benzyladenine, and Thidiazuron, along with nutrients such as ZnSO<sub>4</sub> and KCl. The study aims to assess the phenological effects of the interactions between these cytokinins and nutrients on chickpea crop under water deficit stress.

## 2. Material and Methods

The experiment was conducted during the *Rabi* seasons of 2021-2022 and 2022-2023 at the Experimental Research Farm, Seed Technology Research Unit, JNKVV, Jabalpur (M.P), situated at a latitude of 23°12' N and a longitude of 79°56' E, is approximately 390 meters above mean sea level, with 'Vertisol' soil classification based on U.S. standards. The experiment was carried out using a split-split plot design with three replications, involving the chickpea varieties JG 36 (V<sub>1</sub>) and JG 14 (V<sub>2</sub>) released by Jawaharlal Nehru Krishi Viswa Vidyalyaya, Jabalpur (M.P). Water deficit conditions were assessed using soil moisture content data (at 10-day intervals) and soil water potential data (daily basis).

During the water deficit condition, the foliar spray of plant growth regulators and nutrients was applied at the 50% flowering stage for both D<sub>1</sub> - Control (Irrigation at 30 DAS (Days after sowing) and flower initiation) and D<sub>2</sub> - Drought (Drought stress at flowering up to physiological maturity). During the flowering stage, irrigation was provided only to D<sub>1</sub>. Water stress was imposed in D<sub>2</sub> during the reproductive stage. In both conditions, different plant growth regulators and nutrients were applied at the 50% flowering stage. The details of the PGRs and nutrients are given in Table 1.

**Table 1. Details of plant growth regulators and nutrients used in this study.**

|                |                          |
|----------------|--------------------------|
| T <sub>1</sub> | Control (no spray)       |
| T <sub>2</sub> | Thiourea (TU) @ 1000 ppm |

|                 |                                      |
|-----------------|--------------------------------------|
| T <sub>3</sub>  | Benzyladenine (BA) @ 40 ppm          |
| T <sub>4</sub>  | Thidiazuron (TDZ) @ 10 ppm           |
| T <sub>5</sub>  | 1% ZnSO <sub>4</sub> (Zinc Sulphate) |
| T <sub>6</sub>  | 1% KCl (Potassium Chloride)          |
| T <sub>7</sub>  | TU @ 1000 ppm + 1% ZnSO <sub>4</sub> |
| T <sub>8</sub>  | BA @ 40 ppm + 1% ZnSO <sub>4</sub>   |
| T <sub>9</sub>  | TDZ @ 10 ppm + 1% ZnSO <sub>4</sub>  |
| T <sub>10</sub> | TU @ 1000 ppm + 1% KCl               |
| T <sub>11</sub> | BA @ 40 ppm+ 1% KCl                  |
| T <sub>12</sub> | TDZ @ 10 ppm + 1% KCl                |

For phenological observations, five plants were tagged in each treatment, and the phenological data were recorded by monitoring selected and labeled plants on daily basis throughout the growth cycle.

### 3. Statistical Analysis

The statistical analysis, specifically two-way ANOVA and Tukey's Honest Significant Difference (HSD) test at a 5% level of significance, was performed using R 4.2.2 statistical software. Treatment effects were assessed through analysis of variance using the Split-Split plot design (Gomez and Gomez, 1984). The results for the years 2021-2022, 2022-2023, and the pooled mean were expressed as the average of three replications. Along with the mean values of each treatment Standard error mean (SEm $\pm$ ) and standard deviation (SD) were also mentioned along with the mean value (Table 3 and Table 5). The standard error bars shown in Figure 2 and Figure 4 represents the standard error of the mean.

## 4. Results and Discussion

### 4.1 Effect of foliar spray of plant growth regulators and nutrients on days to pod formation and days to seed formation of chickpea varieties under water deficit stress

The analysis of variance on pooled data revealed significant variation in number of days to pod formation and days to seed formation (Table 2). The range for days to pod formation was found to be from 78.56 to 84.44 DAS (Table 3 and Figure 2). Among the irrigation levels, D<sub>1</sub> (Irrigation at 30 DAS and flower initiation) required

the highest (81.57 DAS) number of days for pod formation followed by D<sub>2</sub> (Drought stress at flowering up to physiological maturity) at 79.27 DAS. Sachdeva *et al.* (2022), indicated that water stress during the reproductive stage leads to a significant reduction in both plant yield and yield-related traits.

Among the varieties, V<sub>1</sub> (JG36) took the longest time (84.44 DAS) days for pod formation, while V<sub>2</sub> (JG 14) required 76.40 DAS. The variation in the time required for pod formation within varieties is likely influenced by a combination of genetic factors, physiological processes, and environmental conditions.

Among the treatments, it was observed that foliar application of TDZ @ 10 ppm + 1% KCl (T<sub>12</sub>) took the longest time (82.56 DAS) for pod formation which is at par with treatment T<sub>9</sub> (TDZ @ 10 ppm + 1% ZnSO<sub>4</sub>) - 81.86 DAS. Conversely, T<sub>1</sub> (Control) achieved pod formation in the shortest time (78.56 DAS), which is at par with T<sub>3</sub> (BA @ 40 ppm) – 79.29 DAS and T<sub>8</sub> (BA @ 40 ppm + 1% ZnSO<sub>4</sub>) – 79.95 DAS. Thidiazuron (TDZ) stimulates cell division, anti-senescence, and anti-stress activity, promotes ethylene production, enhances fruit growth, prolongs flower freshness, and improves stress tolerance and yield in crops (Nisler, 2018). Our result is similar to Cho *et al.* (2022), who reported that the application of thidiazuron, a cytokinin analogue, increases the duration of the vegetative phase by delaying the expression of florigen genes in rice and it also delays rice flowering when the chemical is applied at the floral transition time.

The range for days to seed formation was found to be from 83.45 DAS to 89.86 DAS (Table 3). Among the irrigation levels, the highest (87.10 DAS) number of days to attain seed formation was recorded in D<sub>1</sub> (Irrigation at 30 DAS and flower initiation), followed by D<sub>2</sub> (Drought stress at flowering up to physiological maturity) at 84.02 DAS. Our results are similar to those of Morales *et al.* (2013), who stated that under drought stress, plants exhibited earlier flowering and a shorter fruit production period in raspberry cultivars compared to well-watered plants.

Among the varieties, V<sub>1</sub> (JG36) took the longest time (89.86 DAS) days for seed formation, with V<sub>2</sub> (JG 14) requiring 81.25 DAS. Among the treatments, it was observed that foliar application of TDZ @ 10 ppm + 1% KCl (T<sub>12</sub>) took the longest time (87.67 DAS) for seed formation which is at par with treatment T<sub>4</sub> (TDZ @ 10 ppm) – 86.73 DAS and T<sub>9</sub> (TDZ @ 10 ppm + 1% ZnSO<sub>4</sub>) – 87.22 DAS. The control

treatment, T<sub>1</sub>, achieved seed formation in the shortest time (83.45 DAS), which is at par with T<sub>3</sub> (BA @ 40 ppm) – 84.77 DAS, T<sub>6</sub> (1% KCl) – 84.55 DAS and T<sub>8</sub> (BA @ 40 ppm + 1% ZnSO<sub>4</sub>) – 84.70 DAS. Our results are similar to those of Ferrante et al. (2002) who stated that the application of TDZ has proven effective in delaying both flower and leaf senescence in cut flowers and potted plants. Also, the application of TDZ during the flowering stage of fruit trees has been reported to enhance fruit set and yield in both apple (Jose et al., 2020) and pear (Pasa et al., 2017).

The range of seed filling duration was found to be from 17.27 to 20.89 days (Table 3). Seed filling duration varied significantly among the varied irrigation levels, varieties, foliar spray of plant growth regulators, and nutrients in chickpea (Table 2). Among the irrigation levels, D<sub>1</sub> required more (20.60 days) duration for seed filling followed by D<sub>2</sub> at 17.55 days. Among the varieties, V<sub>1</sub> (JG36) took more time (20.89 days) to seed filling, while V<sub>2</sub> (JG 14) required 17.27 days.

Among the treatments, it was observed that foliar application of TDZ @ 10 ppm + 1% KCl (T<sub>12</sub>) took the longest time (20.30 days) for seed filling which is at par with all other treatments except control T<sub>1</sub> (Control), whereas untreated control required lesser (17.71 days) time for seed filling. This highlights the potential of TDZ to positively influence the seed development process by affecting histogenesis, seed filling, and seed maturation. Our results are similar to those of Liu et al. (2017), who reported that thidiazuron decreased the duration of the rapid increase period and moderately increased the period of the seed-filling stage.

**Table 2. Results of the two-way ANOVA and HSD test for the comparative effects of irrigation levels, varieties, plant growth regulators, and nutrients, on days to pod formation, seed formation, and seed filling duration of Chickpea under water deficit stress.**

| Treatments       | Days to pod formation |           |         | Days to seed formation |                    |                    | Seed filling duration |                    |                    |
|------------------|-----------------------|-----------|---------|------------------------|--------------------|--------------------|-----------------------|--------------------|--------------------|
|                  | 2021-2022             | 2022-2023 | Pooled  | 2021-2022              | 2022-2023          | Pooled             | 2021-2022             | 2022-2023          | Pooled             |
| <b>D</b>         | 0.39*** <sup>a</sup>  | 0.49**    | 0.05*** | 1.11**                 | 1.38*              | 1.14**             | 1.14                  | 0.73**             | 0.78**             |
| <b>V</b>         | 0.40***               | 0.35***   | 0.32*** | 0.93***                | 0.79***            | 0.22***            | 1.22***               | 0.56***            | 0.79***            |
| <b>T</b>         | 0.91***               | 1.00***   | 0.59*** | 1.18***                | 1.19***            | 0.80***            | 2.71*                 | 1.56 <sup>ns</sup> | 1.38*              |
| <b>D x V</b>     | 0.47**                | 0.47**    | 0.32**  | 1.17**                 | 1.19**             | 0.77*              | 1.42**                | 0.73***            | 0.94***            |
| <b>D x T</b>     | 1.28**                | 1.40*     | 0.87*** | 1.69 <sup>ns</sup>     | 1.75 <sup>ns</sup> | 1.24 <sup>ns</sup> | 3.67 <sup>ns</sup>    | 2.13 <sup>ns</sup> | 1.91 <sup>ns</sup> |
| <b>D x V x T</b> | 1.79**                | 1.95*     | 1.19*** | 2.38 <sup>ns</sup>     | 2.42 <sup>ns</sup> | 1.62 <sup>ns</sup> | 5.22 <sup>ns</sup>    | 2.99 <sup>ns</sup> | 2.70 <sup>ns</sup> |

<sup>a</sup> F-values. ns: not significant F ratio ( $p < 0.05$ ); \*, \*\* and \*\*\* indicates significance at  $P < 0.05$ , 0.01 and 0.001, respectively.



**D<sub>1</sub> (Irrigation at 30 DAS and flower initiation)**



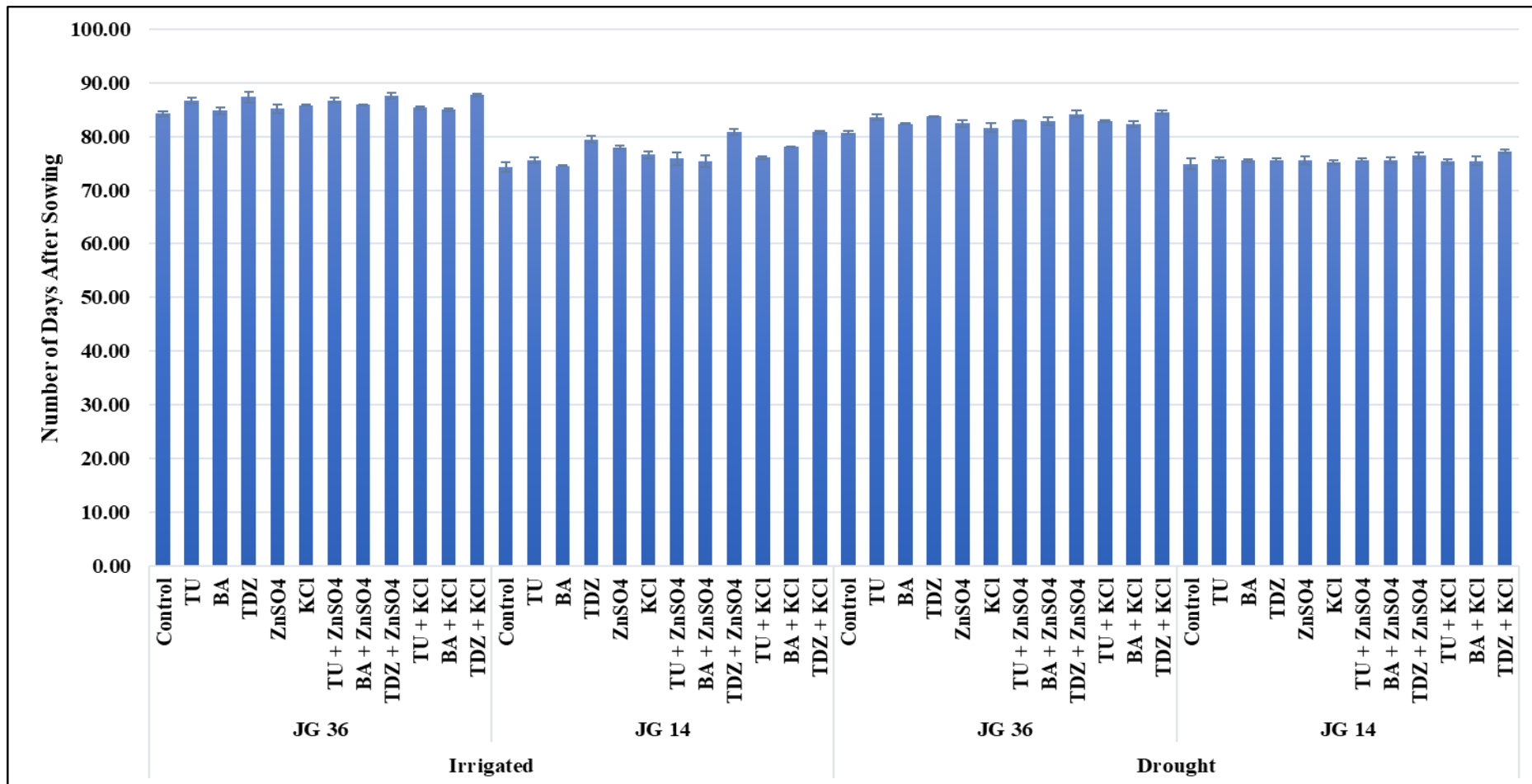
**D<sub>2</sub> (Drought stress at flowering up to physiological maturity)**

**Fig 1. Seed filling stage of Chickpea varieties under irrigated and water deficit stress conditions.**

Table 3. Days to pod formation, days to seed formation, and **seed** filling duration as affected by varied irrigation levels, varieties, foliar spray of plant growth regulators, and nutrients in chickpea

| Treatments                          | Days to Pod formation |                     |                     | Days to Seed formation |                       |                     | Seed filling duration (Days) |                    |                     |
|-------------------------------------|-----------------------|---------------------|---------------------|------------------------|-----------------------|---------------------|------------------------------|--------------------|---------------------|
|                                     | 2021-2022             | 2022-2023           | Pooled              | 2021-2022              | 2022-2023             | Pooled              | 2021-2022                    | 2022-2023          | Pooled              |
| <b>Main plot: Irrigation (D)</b>    |                       |                     |                     |                        |                       |                     |                              |                    |                     |
| D <sub>1</sub>                      | 81.46 <sup>a</sup>    | 81.68 <sup>a</sup>  | 81.57 <sup>a</sup>  | 87.67 <sup>a</sup>     | 86.54 <sup>a</sup>    | 87.10 <sup>a</sup>  | 21.00 <sup>a</sup>           | 20.20 <sup>a</sup> | 20.60 <sup>a</sup>  |
| D <sub>2</sub>                      | 78.49 <sup>b</sup>    | 80.05 <sup>b</sup>  | 79.27 <sup>b</sup>  | 83.15 <sup>b</sup>     | 84.88 <sup>b</sup>    | 84.02 <sup>b</sup>  | 19.95 <sup>a</sup>           | 15.16 <sup>b</sup> | 17.55 <sup>b</sup>  |
| <b>SEm±</b>                         | 0.06                  | 0.08                | 0.009               | 0.18                   | 0.23                  | 0.19                | 0.19                         | 0.12               | 0.13                |
| <b>SD</b>                           | 0.09                  | 0.11                | 0.01                | 0.26                   | 0.32                  | 0.26                | 0.26                         | 0.17               | 0.18                |
| <b>Subplot: Varieties (V)</b>       |                       |                     |                     |                        |                       |                     |                              |                    |                     |
| V <sub>1</sub>                      | 84.01 <sup>a</sup>    | 84.87 <sup>a</sup>  | 84.44 <sup>a</sup>  | 89.94 <sup>a</sup>     | 89.79 <sup>a</sup>    | 89.86 <sup>a</sup>  | 22.40 <sup>a</sup>           | 19.37 <sup>a</sup> | 20.89 <sup>a</sup>  |
| V <sub>2</sub>                      | 75.95 <sup>b</sup>    | 76.86 <sup>b</sup>  | 76.40 <sup>b</sup>  | 80.87 <sup>b</sup>     | 81.62 <sup>b</sup>    | 81.25 <sup>b</sup>  | 18.55 <sup>b</sup>           | 15.99 <sup>b</sup> | 17.27 <sup>b</sup>  |
| <b>SEm±</b>                         | 0.10                  | 0.09                | 0.08                | 0.24                   | 0.20                  | 0.05                | 0.31                         | 0.14               | 0.20                |
| <b>SD</b>                           | 0.14                  | 0.13                | 0.11                | 0.33                   | 0.28                  | 0.08                | 0.44                         | 0.20               | 0.28                |
| <b>Sub-sub plot: Treatments (T)</b> |                       |                     |                     |                        |                       |                     |                              |                    |                     |
| T <sub>1</sub>                      | 77.81 <sup>f</sup>    | 79.32 <sup>c</sup>  | 78.56 <sup>e</sup>  | 83.92 <sup>d</sup>     | 82.98 <sup>e</sup>    | 83.45 <sup>d</sup>  | 17.65 <sup>b</sup>           | 17.77 <sup>a</sup> | 17.71 <sup>b</sup>  |
| T <sub>2</sub>                      | 79.99 <sup>cde</sup>  | 80.79 <sup>bc</sup> | 80.39 <sup>c</sup>  | 85.42 <sup>abcd</sup>  | 85.37 <sup>cd</sup>   | 85.39 <sup>bc</sup> | 19.99 <sup>ab</sup>          | 17.85 <sup>a</sup> | 18.92 <sup>ab</sup> |
| T <sub>3</sub>                      | 78.71 <sup>ef</sup>   | 79.86 <sup>c</sup>  | 79.29 <sup>de</sup> | 84.08 <sup>d</sup>     | 85.46 <sup>cd</sup>   | 84.77 <sup>cd</sup> | 19.07 <sup>ab</sup>          | 17.68 <sup>a</sup> | 18.37 <sup>ab</sup> |
| T <sub>4</sub>                      | 80.90 <sup>abc</sup>  | 82.17 <sup>ab</sup> | 81.54 <sup>b</sup>  | 86.25 <sup>abc</sup>   | 87.21 <sup>abc</sup>  | 86.73 <sup>ab</sup> | 22.14 <sup>ab</sup>          | 17.36 <sup>a</sup> | 19.75 <sup>ab</sup> |
| T <sub>5</sub>                      | 79.80 <sup>cde</sup>  | 80.76 <sup>bc</sup> | 80.28 <sup>c</sup>  | 85.42 <sup>abcd</sup>  | 85.74 <sup>bcd</sup>  | 85.58 <sup>bc</sup> | 20.29 <sup>ab</sup>          | 17.09 <sup>a</sup> | 18.69 <sup>ab</sup> |
| T <sub>6</sub>                      | 79.52 <sup>cde</sup>  | 80.11 <sup>c</sup>  | 79.81 <sup>cd</sup> | 84.58 <sup>cd</sup>    | 84.51 <sup>de</sup>   | 84.55 <sup>cd</sup> | 21.97 <sup>ab</sup>          | 17.78 <sup>a</sup> | 19.87 <sup>ab</sup> |
| T <sub>7</sub>                      | 80.38 <sup>bcd</sup>  | 80.26 <sup>c</sup>  | 80.32 <sup>c</sup>  | 85.50 <sup>abcd</sup>  | 86.08 <sup>abcd</sup> | 85.79 <sup>bc</sup> | 20.08 <sup>ab</sup>          | 17.94 <sup>a</sup> | 19.01 <sup>ab</sup> |
| T <sub>8</sub>                      | 79.82 <sup>cde</sup>  | 80.09 <sup>c</sup>  | 79.95 <sup>de</sup> | 85.17 <sup>bcd</sup>   | 84.24 <sup>de</sup>   | 84.70 <sup>cd</sup> | 20.63 <sup>ab</sup>          | 17.50 <sup>a</sup> | 19.07 <sup>ab</sup> |
| T <sub>9</sub>                      | 81.86 <sup>ab</sup>   | 82.70 <sup>a</sup>  | 82.28 <sup>ab</sup> | 86.75 <sup>ab</sup>    | 87.69 <sup>ab</sup>   | 87.22 <sup>a</sup>  | 21.91 <sup>ab</sup>          | 17.18 <sup>a</sup> | 19.55 <sup>ab</sup> |
| T <sub>10</sub>                     | 79.25 <sup>def</sup>  | 80.54 <sup>bc</sup> | 79.90 <sup>cd</sup> | 85.42 <sup>abcd</sup>  | 85.35 <sup>cd</sup>   | 85.39 <sup>bc</sup> | 20.17 <sup>ab</sup>          | 17.88 <sup>a</sup> | 19.03 <sup>ab</sup> |
| T <sub>11</sub>                     | 79.66 <sup>cde</sup>  | 80.74 <sup>bc</sup> | 80.20 <sup>cd</sup> | 85.17 <sup>bcd</sup>   | 85.78 <sup>bcd</sup>  | 85.48 <sup>bc</sup> | 19.47 <sup>ab</sup>          | 17.89 <sup>a</sup> | 18.68 <sup>ab</sup> |
| T <sub>12</sub>                     | 82.05 <sup>a</sup>    | 83.07 <sup>a</sup>  | 82.56 <sup>a</sup>  | 87.25 <sup>a</sup>     | 88.09 <sup>a</sup>    | 87.67 <sup>a</sup>  | 22.37 <sup>a</sup>           | 18.23 <sup>a</sup> | 20.30 <sup>a</sup>  |
| <b>SEm±</b>                         | 0.32                  | 0.36                | 0.21                | 0.42                   | 0.42                  | 0.28                | 0.96                         | 0.55               | 0.49                |
| <b>SD</b>                           | 0.46                  | 0.51                | 0.29                | 0.59                   | 0.60                  | 0.40                | 1.36                         | 0.78               | 0.69                |

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



TU – Thiourea @ 1000 ppm, BA – Benzyladenine @ 40 ppm, TDZ - Thidiazuron @ 10 ppm, ZnSO4 - 1% ZnSO4, KCl - 1% KCl. **The error bars shown in the figure represent the standard error of the mean.**

**Fig 2. Effect of foliar spray of plant growth regulators and nutrients on days to pod formation of chickpea varieties under water deficit stress conditions.**

## 4.2 Effect of foliar spray of plant growth regulators and nutrients on days to physiological maturity and days to harvest maturity of chickpea varieties under water deficit stress

The results from the pooled analysis of two consecutive years indicated significant variation in the number of days to physiological maturity and days to harvest maturity (Table 4).

The range for days to physiological maturity was found to be from 96.27 DAS to 105.33 DAS (Table 5). Among the irrigation levels, the highest (102.18 DAS) number of days to attain physiological maturity was recorded in D<sub>1</sub> (Irrigation at 30 DAS and flower initiation), followed by D<sub>2</sub> (Drought stress at flowering up to physiological maturity) at 96.93 DAS. This might be due to that when a plant faces water deficit stress, it tends to translocate its resources as a survival strategy. In conditions of limited water availability, the plant may prioritize reproductive processes, including flowering and fruiting, over vegetative growth. This reallocation of resources toward reproductive structures can expedite the onset of maturity.

With respect to varieties, JG 36 (V<sub>1</sub>) required more time (105.33 DAS) to attain physiological maturity, whereas JG 14 (V<sub>2</sub>) reached physiological maturity earlier (96.67 DAS). With respect to the application of plant growth regulators and nutrients, treatment T<sub>12</sub> (TDZ @ 10 ppm + 1% KCl) required more time (102.86 DAS) to attain physiological maturity which is at par with T<sub>4</sub> (TDZ @ 10 ppm) – 101.29 DAS and T<sub>9</sub> (TDZ @ 10 ppm + 1% ZnSO<sub>4</sub>) - 101.83 DAS. Physiological maturity was observed earlier (96.27 DAS) in control (T<sub>1</sub>), which is at par with T<sub>3</sub> (BA @ 40 ppm) 97.66 DAS.

Toscano *et al.* (2018) stated that the exogenous application of TDZ inhibits leaf senescence by preserving the integrity of leaf pigments, maintaining membrane integrity, and sustaining antioxidant activity, while also keeping a basal level of ABA. The synthetic analogs of cytokinins, specifically thidiazuron (TDZ), have been documented to delay the initiation of leaf senescence in *Pelargonium* sp. (Currey and Lopez, 2013; Mutui *et al.*, 2012). Additionally, TDZ has been reported to reduce the senescence of both leaves and flower abscission in cut inflorescences of phlox and lupins (Sankhla *et al.*, 2003).

The range for days to harvest maturity was found to be from 105.45 DAS to 113.51 DAS (Table 5 and Figure 4). With respect to irrigation levels, harvest maturity was observed to be earlier (106.25 DAS) in D<sub>2</sub> (Drought stress at flowering up to physiological maturity), whereas D<sub>1</sub> (Irrigation at 30 DAS and flower initiation) showed delayed (113.51 DAS) harvest maturity. This may be due to that under water deficit stress, plants prioritize reproductive processes, hastening maturity for efficient resource allocation. Reduced photosynthetic rates prompt early maturation to complete reproduction. Early maturity serves as an adaptive mechanism for life cycle completion in challenging environments. Water-stressed plants conserve energy by expediting maturation for optimal resource use. The phenomenon observed in this study coincides with the findings of Mc Vay *et al.* (2013), who noted that the chickpea crop hastens maturity by stopping plant growth under water deficit stress conditions.

Among the varieties, JG 36 (V<sub>1</sub>) achieved harvest maturity later (112.90 DAS) whereas JG 14 (V<sub>2</sub>) recorded an earlier (106.86) harvest maturity. This might be due to JG 14 exhibiting accelerated early maturity beyond its typical early maturation compared to irrigated treatment. The difference in harvest maturity among JG 36 (V<sub>1</sub>) and JG 14 (V<sub>2</sub>) is due to genetic variations in physiological processes, including photosynthesis and water uptake due to water deficit stress. The observed early maturity in JG 14 under water deficit highlights the impact of environmental stress on varietal responses.

In terms of the application of plant growth regulators and nutrients, treatment T<sub>12</sub> (TDZ @ 10 ppm + 1% KCl) required more time (114.07 DAS) to attain harvest maturity, while the control (T<sub>1</sub>) achieved an early (105.45 DAS) harvest maturity. This may be due to the application of thidiazuron, which delays crop maturity due to its anti-senescence properties, inhibition of ethylene synthesis, stimulation of cell division, and regulation of floral transition. These effects collectively contribute to a prolonged growth period and delay in crop maturity. Our results are similar to Ferrante *et al.* (2002), who reported that the application of TDZ extended leaf longevity, delayed leaf senescence, and crop maturity.

**Table 4. Results of the two-way ANOVA and HSD test for the comparative effects of plant growth regulators and nutrients, irrigation levels, and varieties on days to physiological maturity and harvest maturity of Chickpea under water deficit stress.**

| Treatments       | Days to physiological maturity |                     |                     | Days to harvest maturity |                     |                     |
|------------------|--------------------------------|---------------------|---------------------|--------------------------|---------------------|---------------------|
|                  | 2021-2022                      | 2022-2023           | Pooled              | 2021-2022                | 2022-2023           | Pooled              |
| <b>D</b>         | 1.23 <sup>***a</sup>           | 0.35 <sup>***</sup> | 0.79 <sup>**</sup>  | 0.60 <sup>***</sup>      | 2.22 <sup>**</sup>  | 1.13 <sup>**</sup>  |
| <b>V</b>         | 1.09 <sup>***</sup>            | 0.45 <sup>***</sup> | 0.64 <sup>***</sup> | 0.57 <sup>***</sup>      | 0.65 <sup>***</sup> | 0.36 <sup>***</sup> |
| <b>T</b>         | 2.51 <sup>***</sup>            | 1.00 <sup>***</sup> | 1.24 <sup>***</sup> | 0.84 <sup>***</sup>      | 1.34 <sup>***</sup> | 0.82 <sup>***</sup> |
| <b>D x V</b>     | 1.35 <sup>***</sup>            | 0.50 <sup>***</sup> | 0.81 <sup>***</sup> | 0.69                     | 1.57 <sup>***</sup> | 0.81 <sup>***</sup> |
| <b>D x T</b>     | 3.41 <sup>ns</sup>             | 1.40 <sup>ns</sup>  | 1.73 <sup>ns</sup>  | 1.21 <sup>***</sup>      | 2.09 <sup>**</sup>  | 1.27 <sup>***</sup> |
| <b>D x V x T</b> | 4.84 <sup>ns</sup>             | 1.96 <sup>ns</sup>  | 2.42 <sup>ns</sup>  | 1.68 <sup>**</sup>       | 2.77 <sup>ns</sup>  | 1.67 <sup>*</sup>   |

<sup>a</sup> F-values. ns: not significant F ratio ( $p < 0.05$ ); \*, \*\* and \*\*\* indicates significance at  $P < 0.05$ ,  $0.01$  and  $0.001$ , respectively.

**Table 5. Physiological maturity and Harvest maturity as affected by varied irrigation levels, varieties, foliar spray of plant growth regulators, and nutrients in Chickpea**

| Treatments                          | Days to Physiological maturity |                      |                      | Days to Harvest maturity |                       |                       |
|-------------------------------------|--------------------------------|----------------------|----------------------|--------------------------|-----------------------|-----------------------|
|                                     | 2021-2022                      | 2022-2023            | Pooled               | 2021-2022                | 2022-2023             | Pooled                |
| <b>Main plot: Irrigation (D)</b>    |                                |                      |                      |                          |                       |                       |
| D <sub>1</sub>                      | 102.47 <sup>a</sup>            | 101.89 <sup>a</sup>  | 102.18 <sup>a</sup>  | 112.38 <sup>a</sup>      | 114.65 <sup>a</sup>   | 113.51 <sup>a</sup>   |
| D <sub>2</sub>                      | 98.44 <sup>b</sup>             | 95.21 <sup>b</sup>   | 96.83 <sup>b</sup>   | 106.98 <sup>b</sup>      | 105.51 <sup>b</sup>   | 106.25 <sup>b</sup>   |
| <b>SEm±</b>                         | 0.20                           | 0.06                 | 0.13                 | 0.09                     | 0.36                  | 0.19                  |
| <b>SD</b>                           | 0.29                           | 0.08                 | 0.18                 | 0.14                     | 0.51                  | 0.26                  |
| <b>Subplot: Varieties (V)</b>       |                                |                      |                      |                          |                       |                       |
| V <sub>1</sub>                      | 106.42 <sup>a</sup>            | 104.24 <sup>a</sup>  | 105.33 <sup>a</sup>  | 113.19 <sup>a</sup>      | 112.61 <sup>a</sup>   | 112.90 <sup>a</sup>   |
| V <sub>2</sub>                      | 94.50 <sup>b</sup>             | 92.85 <sup>b</sup>   | 96.67 <sup>b</sup>   | 106.17 <sup>b</sup>      | 107.55 <sup>b</sup>   | 106.86 <sup>b</sup>   |
| <b>SEm±</b>                         | 0.28                           | 0.11                 | 0.16                 | 0.14                     | 0.16                  | 0.09                  |
| <b>SD</b>                           | 0.39                           | 0.16                 | 0.23                 | 0.21                     | 0.23                  | 0.13                  |
| <b>Sub-sub plot: Treatments (T)</b> |                                |                      |                      |                          |                       |                       |
| T <sub>1</sub>                      | 95.45 <sup>e</sup>             | 97.09 <sup>d</sup>   | 96.27 <sup>d</sup>   | 104.40 <sup>f</sup>      | 106.50 <sup>e</sup>   | 105.45 <sup>g</sup>   |
| T <sub>2</sub>                      | 99.98 <sup>bcd</sup>           | 98.64 <sup>bcd</sup> | 99.31 <sup>bc</sup>  | 109.35 <sup>cd</sup>     | 109.80 <sup>bcd</sup> | 109.57 <sup>def</sup> |
| T <sub>3</sub>                      | 97.78 <sup>de</sup>            | 97.54 <sup>d</sup>   | 97.66 <sup>cd</sup>  | 107.56 <sup>e</sup>      | 109.27 <sup>cd</sup>  | 108.41 <sup>f</sup>   |
| T <sub>4</sub>                      | 103.04 <sup>abc</sup>          | 99.53 <sup>bc</sup>  | 101.29 <sup>ab</sup> | 111.92 <sup>b</sup>      | 111.33 <sup>bc</sup>  | 111.62 <sup>bc</sup>  |
| T <sub>5</sub>                      | 100.10 <sup>bcd</sup>          | 97.85 <sup>cd</sup>  | 98.97 <sup>c</sup>   | 108.53 <sup>de</sup>     | 108.76 <sup>de</sup>  | 108.65 <sup>ef</sup>  |
| T <sub>6</sub>                      | 101.49 <sup>abcd</sup>         | 97.88 <sup>cd</sup>  | 99.69 <sup>bc</sup>  | 109.49 <sup>cd</sup>     | 108.90 <sup>d</sup>   | 109.19 <sup>def</sup> |
| T <sub>7</sub>                      | 100.45 <sup>abcd</sup>         | 98.21 <sup>bcd</sup> | 99.33 <sup>bc</sup>  | 109.43 <sup>cd</sup>     | 109.47 <sup>cd</sup>  | 109.45 <sup>def</sup> |
| T <sub>8</sub>                      | 100.44 <sup>abcd</sup>         | 97.60 <sup>d</sup>   | 99.02 <sup>c</sup>   | 110.07 <sup>c</sup>      | 109.58 <sup>cd</sup>  | 109.82 <sup>de</sup>  |
| T <sub>9</sub>                      | 103.77 <sup>ab</sup>           | 99.89 <sup>ab</sup>  | 101.83 <sup>a</sup>  | 112.16 <sup>b</sup>      | 111.97 <sup>b</sup>   | 112.07 <sup>b</sup>   |
| T <sub>10</sub>                     | 99.42 <sup>cde</sup>           | 98.42 <sup>bcd</sup> | 98.92 <sup>c</sup>   | 109.65 <sup>cd</sup>     | 110.03 <sup>bcd</sup> | 109.84 <sup>de</sup>  |
| T <sub>11</sub>                     | 99.13 <sup>cde</sup>           | 98.62 <sup>bcd</sup> | 98.88 <sup>c</sup>   | 109.81 <sup>cd</sup>     | 110.97 <sup>bcd</sup> | 110.39 <sup>cd</sup>  |
| T <sub>12</sub>                     | 104.42 <sup>a</sup>            | 101.30 <sup>a</sup>  | 102.86 <sup>a</sup>  | 113.80 <sup>a</sup>      | 114.34 <sup>a</sup>   | 114.07 <sup>a</sup>   |
| <b>SEm±</b>                         | 0.89                           | 0.36                 | 0.44                 | 0.30                     | 0.48                  | 0.29                  |
| <b>SD</b>                           | 1.26                           | 0.50                 | 0.62                 | 0.42                     | 0.68                  | 0.41                  |

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



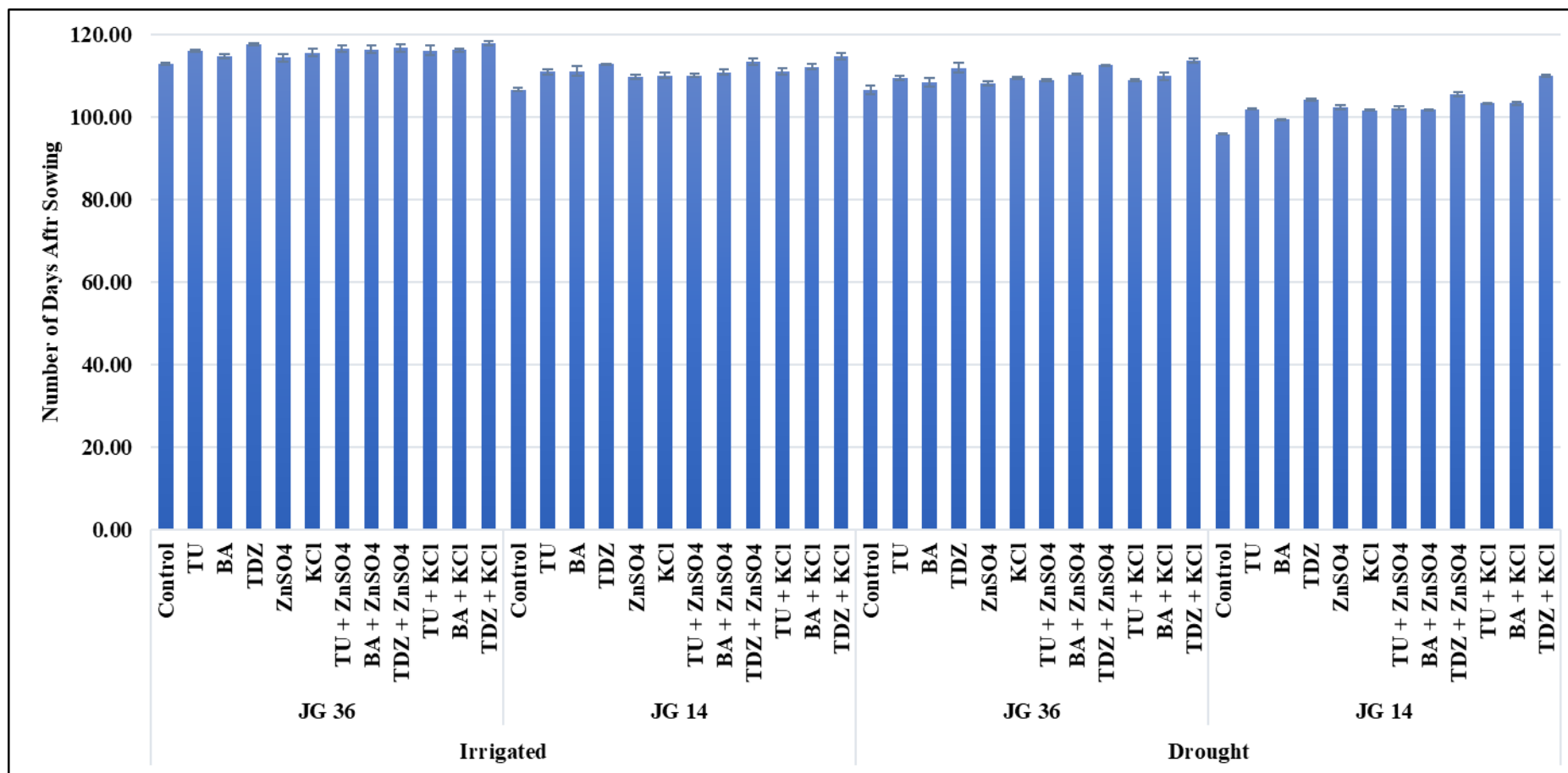
**V<sub>1</sub> - JG 36 (Late maturity)**



**V<sub>2</sub> - JG 14 (Early maturity)**

**Fig 3. Physiological maturity stage of Chickpea JG 36 and JG 14 varieties**

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TU – Thiourea @ 1000 ppm, BA – Benzyladenine @ 40 ppm, TDZ - Thidiazuron @ 10 ppm, ZnSO<sub>4</sub>- 1% ZnSO<sub>4</sub>, KCl - 1% KCl. The error bars shown in the figure represent the standard error of the mean.

**Fig 4. Effect of foliar spray of plant growth regulators and nutrients on days to harvest maturity of chickpea varieties under water deficit stress**

## Conclusion

Among the different irrigation levels, well-irrigated plants exhibited delayed maturity, while water-stressed plants showed an early onset of maturity, indicating resource reallocation as a survival strategy. Late-maturing varieties like JG 36 may be better suited to environments with longer growing seasons, allowing them to utilize available resources over an extended period. In contrast, early-maturing varieties such as JG 14 may have adaptations that enable them to complete their reproductive stages more quickly, making them suitable for regions with limited water availability.

TDZ @ 10 ppm + 1% KCl significantly influenced phenological stages *i.e.*, delayed both pod and seed formation as well as physiological and harvest maturity. This showed the potential of TDZ to positively influence the seed development process by affecting histogenesis, seed filling, and seed maturation. The combination of cytokinin analogs and nutrients proved to be a novel approach, offering insights for sustainable chickpea cultivation under water deficit conditions. Further investigations are needed to identify the impact of TDZ and KCl in enhancing seed yield and seed weight of chickpeas under optimal and sub-optimal soil water conditions, and to provide recommendations for chickpea growers.

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