Phenological Variation in Chickpea through Foliar Application of Cytokinin Analogs and Nutrients Under Water Deficit Stress

Abstract

Comment [TK1]: Please include research methods

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. This research also investigates the impact of foliar applications of cytokinin analogs *viz*., Thiourea, Thidiazuron, and Benzyladenine and nutrients *viz.,* $ZnSO_4$ 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_1 (Irrigation at 30 DAS and at flower initiation) exhibited delayed phenological stages of the crop, while D_2 (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_{12} (TDZ @ 10 ppm + 1% KCl) significantly delayed pod and seed formation, as well as physiological and harvest maturity as compared to untreated control (T_1). Foliar application of TDZ @ 10 ppm + 1% KCl (T_{12}) 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: Chickpea; Phenology; Thidiazuron; Benzyladenine; Water deficit stress.

Comment [TK2]: Please sort alphabetically

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 for 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 is 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 condition, the crop frequently faces the water deficit stress condition especially during the reproductive stage of the crop (Turner *et al*., 2004), accounting 40% - 45% of global chickpea yield losses (Ahmed *et al*., 2005). During the grain-filling stage of the crop, 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 the geographical location (Sachdeva *et al*., 2022). Plants have the ability to 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.,* disrupts the plant's phenology, metabolism, and various physiological processes (Keerthana *et al*., 2022). Berger *et al*., (2003) states that the 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, cytokinin’s (CKs) are known to regulate several aspects of plant growth and development, including the response of plants to abiotic stress (Rivero *et al*., 2007). Cytokinin’s can regulate various physiological processes in plants and also provide protective effects under stress conditions (Vineeth *et al*., 2016).

Thiourea (TU), 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 condition (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).

Under water deficit condition, 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 crop (Hussain *et al*., 2020). Zinc plays a crucial role in the reproductive development of plants, influencing the flower initiation, floral development, as well as male and female gametogenesis, fertilization, and seed development (Pathak *et al*., 2012). Zinc deficiency also results in a 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, have a major role in plant physiological processes such as photosynthesis, assimilate transportation, and activation of enzymes (Hasanuzzaman *et al*., 2018). Application of potassium at vegetative stage of the pulse crop boosts the crop growth rate, yield and yield components under water deficit condition (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 $ZnSO_4$ and KCl. The study aims to assess the phenological effects of the interactions between these cytokinin's 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_1) and JG 14 (V_2) released by Jawaharlal Nehru Krishi Viswa Vidyalaya, Jabalpur (M.P). Water deficit conditions were assessed using soil moisture content data (at 10 days 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_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, T_{12} - 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 a daily basis throughout the growth cycle.

Comment [TK3]: What is DAS? Whatdoes DAS stand for?

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±), Standard deviation (SD) was also mentioned along with mean value (Table 2 and Table 4).

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 1). The range for days to pod formation was found to be from 78.56 to 84.44 DAS (Table 2 and Figure 2). Among the irrigation levels, D_1 (Irrigation at 30 DAS and at flower initiation) required the highest (81.57 DAS) number of days for pod formation followed by D_2 (Drought stress at flowering up to physiological maturity) at 79.27 DAS. Sachdeva *et al*. (2022), indicated that that water stress during the reproductive stage leads to a significant reduction in both plant yield and yield-related traits.

Among the varieties, V_1 (JG36) took the longest time (84.44 DAS) days for pod formation, while V_2 (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 (T12) took the longest time (82.56 DAS) for pod formation which is at par with treatment T_9 (TDZ @ 10 ppm + 1% $ZnSO_4$) - 81.86 DAS. Conversely, T_1 (Control) achieved pod formation in the shortest time (78.56 DAS), which is at par with T_3 (BA @ 40 ppm) – 79.29 DAS and T_8 (BA 40 @ ppm + 1% $ZnSO_4$) – 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 in similar with 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 2). Among the irrigation levels, the highest (87.10 DAS) number of days to attain seed formation was recorded in D_1 (Irrigation at 30 DAS and at flower initiation), followed by D_2 (Drought stress at flowering up to physiological maturity) at 84.02 DAS. Among the varieties, V_1 (JG36) took longest time (89.86 DAS) days for seed formation, with V_2 (JG 14) required 81.25 DAS. Among the treatments, it was observed that foliar application of TDZ @ 10 ppm + 1% KCl (T_{12}) took the longest time (87.67 DAS) for seed formation which is at par with treatment T_4 (TDZ @ 10 ppm) – 86.73 DAS and T_9 (TDZ @ 10 ppm + 1% $ZnSO_4$) – 87.22 DAS. The control treatment, T_1, achieved seed formation in the shortest time (83.45 DAS), which is at par with T_3 (BA @ 40 ppm) – 84.77 DAS, T_6 (1% KCl) – 84.55 DAS and T_8 (BA 40 @ ppm + 1% $ZnSO_4$) – 84.70 DAS. TDZ has proven effective in delaying both flower and leaf senescence in cut flowers and potted plants (Ferrante *et al*., 2002).

Comment [TK4]: Please compare the results of this study with similar studies

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). TDZ treatment has been shown to induce gene expression related to ethylene biosynthesis and signalling, 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). In tulips, cytokinin's were found to delay flower senescence (Van Doorn *et al*., 2011).

Comment [TK5]: Please explain the relationship between these references and the results of TDZ's effect for seed formation.

The range of seed filling duration was found to be from 17.27 to 20.89 days (Table 2). Seed filling duration varied significantly among the varied irrigation levels, varieties, foliar spray of plant growth regulators and nutrients in chickpea (Table 1). Among the irrigation levels, D_1 (Irrigation at 30 DAS and at flower initiation) required more (20.60 days) duration for seed filling followed by D_2 (Drought stress at flowering up to physiological maturity) at 17.55 days. Among the varieties, V_1 (JG36) took more time (20.89 days) to seed filling, while V_2 (JG 14) required 17.27 days.

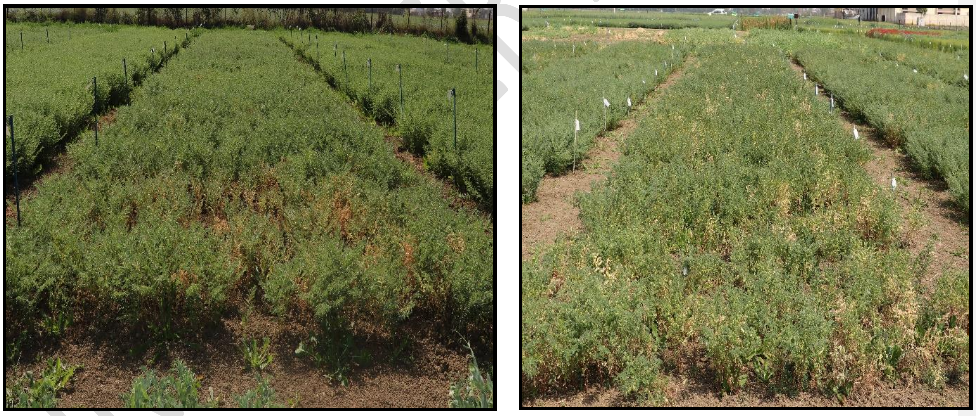
Comment [TK6]: I think for writing just D1 is enough because previously it was written that D1 is "Irrigation at 30 DAS and at flower initiation "with brackets. the same thing also needs to be considered on D2.

Among the treatments, it was observed that foliar application of TDZ @ 10 ppm + 1% KCl (T_{12}) took the longest time (20.30 days) for seed filling which is at par with all other treatments except control T_1 (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 grain-filling stage.

Table 1. 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
D	0.39***[a]	0.49**	0.05***	1.11**	1.38*	1.14**	1.14	0.73**	0.78**
V	0.40***	0.35***	0.32***	0.93***	0.79***	0.22***	1.22***	0.56***	0.79***
T	0.91***	1.00***	0.59***	1.18***	1.19***	0.80***	2.71*	1.56 ns	1.38*
D x V	0.47**	0.47**	0.32**	1.17**	1.19**	0.77*	1.42**	0.73***	0.94***
D x T	1.28**	1.40*	0.87***	1.69 ns	1.75 ns	1.24 ns	3.67 ns	2.13 ns	1.91 ns
D x V x T	1.79**	1.95*	1.19***	2.38 ns	2.42 ns	1.62 ns	5.22 ns	2.99 ns	2.70 ns

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



D_1 (Irrigation at 30 DAS and at flower initiation)

D_2 (Drought stress at flowering up to physiological maturity)

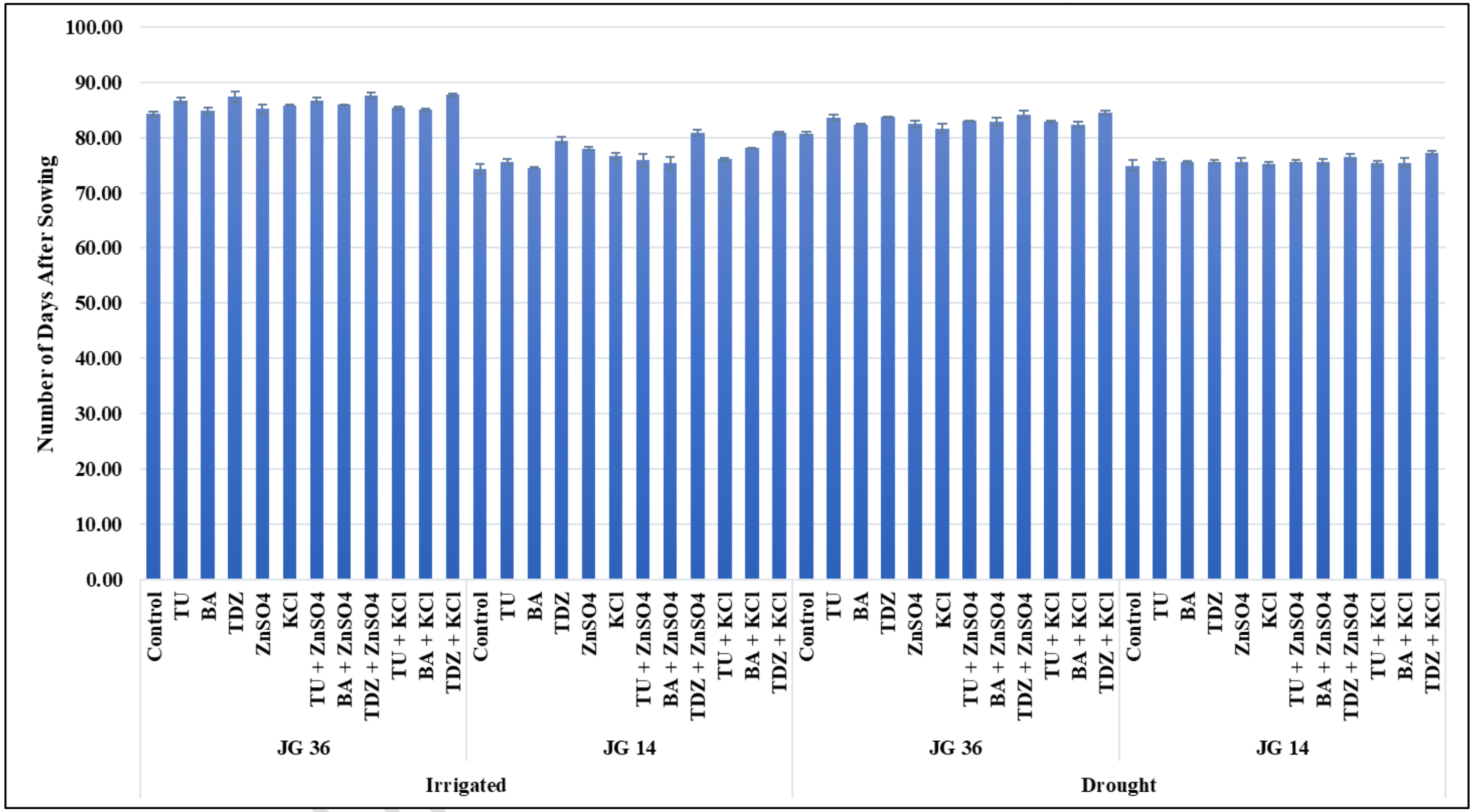
Fig 1. D_1 and D_2 at the seed filling stage

Comment [TK7]: please correct the image title so that it is more informative

Table 2. Days to pod formation, days to seed formation and grain filling duration (days) as affected by varied irrigation level, varieties, foliar spray of plant growth regulators and nutrients in chickpea

	Days to Pod formation			Days to Seed formation			Grain filling duration (Days)		
Treatments	**2021-2022**	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**
Main plot: Irrigation (D)									
D_1	81.46^{a}	81.68^{a}	81.57^{a}	87.67^{a}	86.54^{a}	87.10^{a}	21.00^{a}	20.20^{a}	20.60^{a}
D_2	78.49^{b}	80.05^{b}	79.27^{b}	83.15^{b}	84.88^{b}	84.02^{b}	19.95^{a}	15.16^{b}	17.55^{b}
SEm±	0.06	0.08	0.009	0.18	0.23	0.19	0.19	0.12	0.13
SD	0.09	0.11	0.01	0.26	0.32	0.26	0.26	0.17	0.18
Subplot: Varieties (V)									
V_1	84.01^{a}	84.87^{a}	84.44^{a}	89.94^{a}	89.79^{a}	89.86^{a}	22.40^{a}	19.37^{a}	20.89^{a}
V_2	75.95^{b}	76.86^{b}	76.40^{b}	80.87^{b}	81.62^{b}	81.25^{b}	18.55^{b}	15.99^{b}	17.27^{b}
SEm±	0.10	0.09	0.08	0.24	0.20	0.05	0.31	0.14	0.20
SD	0.14	0.13	0.11	0.33	0.28	0.08	0.44	0.20	0.28
Sub-sub plot: Treatments (T)									
T_1	77.81^{f}	79.32^{c}	78.56^{e}	83.92^{d}	82.98^{e}	83.45^{d}	17.65^{b}	17.77^{a}	17.71^{b}
T_2	79.99^{cde}	80.79^{bc}	80.39^{c}	85.42^{abcd}	85.37^{cd}	85.39^{bc}	19.99^{ab}	17.85^{a}	18.92^{ab}
T_3	78.71^{ef}	79.86^{c}	79.29^{de}	84.08^{d}	85.46^{cd}	84.77^{cd}	19.07^{ab}	17.68^{a}	18.37^{ab}
T_4	80.90^{abc}	82.17^{ab}	81.54^{b}	86.25^{abc}	87.21^{abc}	86.73^{ab}	22.14^{ab}	17.36^{a}	19.75^{ab}
T_5	79.80^{cde}	80.76^{bc}	80.28^{c}	85.42^{abcd}	85.74^{bcd}	85.58^{bc}	20.29^{ab}	17.09^{a}	18.69^{ab}
T_6	79.52^{cde}	80.11^{c}	79.81^{cd}	84.58^{cd}	84.51^{de}	84.55^{cd}	21.97^{ab}	17.78^{a}	19.87^{ab}
T_7	80.38^{bcd}	80.26^{c}	80.32^{c}	85.50^{abcd}	86.08^{abcd}	85.79^{bc}	20.08^{ab}	17.94^{a}	19.01^{ab}
T_8	79.82^{cde}	80.09^{c}	79.95^{de}	85.17^{bcd}	84.24^{de}	84.70^{cd}	20.63^{ab}	17.50^{a}	19.07^{ab}
T_9	81.86^{ab}	82.70^{a}	82.28^{ab}	86.75^{ab}	87.69^{ab}	87.22^{a}	21.91^{ab}	17.18^{a}	19.55^{ab}
T_{10}	79.25^{def}	80.54^{bc}	79.90^{cd}	85.42^{abcd}	85.35^{cd}	85.39^{bc}	20.17^{ab}	17.88^{a}	19.03^{ab}
T_{11}	79.66^{cde}	80.74^{bc}	80.20^{cd}	85.17^{bcd}	85.78^{bcd}	85.48^{bc}	19.47^{ab}	17.89^{a}	18.68^{ab}
T_{12}	82.05^{a}	83.07^{a}	82.56^{a}	87.25^{a}	88.09^{a}	87.67^{a}	22.37^{a}	18.23^{a}	20.30^{a}
SEm±	0.32	0.36	0.21	0.42	0.42	0.28	0.96	0.55	0.49
SD	0.46	0.51	0.29	0.59	0.60	0.40	1.36	0.78	0.69

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



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

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

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

The results from the pooled analysis of two consecutive years indicate significant variation in the number of days to physiological maturity and days to harvest maturity (Table 3).

Comment [TK8]: indicated

The range for days to physiological maturity was found to be from 96.27 DAS to 105.33 DAS (Table 4). Among the irrigation levels, the highest (102.18 DAS) number of days to attain physiological maturity was recorded in D_1 (Irrigation at 30 DAS and at flower initiation), followed by D_2 (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.

Comment [TK9]: please add supporting references

With respect to varieties, JG 36 (V_1) required more time (105.33 DAS) to attain physiological maturity, whereas JG 14 (V_2) reached physiological maturity earlier (96.67 DAS). With respect to the application of plant growth regulators and nutrients, treatment T_{12} (TDZ @ 10 ppm + 1% KCl) required more time (102.86 DAS) to attain physiological maturity which is at par with T_4 (TDZ @ 10 ppm) – 101.29 DAS and T_9 (TDZ @ 10 ppm + 1% $ZnSO_4$) - 101.83 DAS. Physiological maturity was observed earlier (96.27 DAS) in control (T_1), which is at par with T_3 (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 cytokinin's, 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 4 and Figure 4). With respect to irrigation levels, harvest maturity was observed to be earlier (106.25 DAS) in D_2 (Drought stress at flowering up to physiological maturity), whereas D_1 (Irrigation at 30 DAS and at 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_1) achieved harvest maturity later (112.90 DAS) whereas JG 14 (V_2) recorded an earlier (106.86) harvest maturity. This might be due to JG 14 exhibits accelerated early maturity beyond its typical early maturation compared to irrigated treatment. The difference in harvest maturity among JG 36 (V_1) and JG 14 (V_2) 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.

Comment [TK10]: please add supporting references

In terms of the application of plant growth regulators and nutrients, treatment T_{12} (TDZ @ 10 ppm + 1% KCl) required more time (114.07 DAS) to attain harvest maturity, while the control (T_1) achieved an early (105.45 DAS) harvest maturity. This may be due to the application of thidiazuron, delays crop maturity due to its anti-senescence properties, inhibition of ethylene synthesis, simulation 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 3. 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
D	1.23**[a]	0.35***	0.79**	0.60***	2.22**	1.13**
V	1.09***	0.45***	0.64***	0.57***	0.65***	0.36***
T	2.51***	1.00***	1.24***	0.84***	1.34***	0.82***
D x V	1.35***	0.50***	0.81***	0.69	1.57***	0.81***
D x T	3.41^{ns}	1.40^{ns}	1.73^{ns}	1.21***	2.09**	1.27***
D x V x T	4.84^{ns}	1.96^{ns}	2.42^{ns}	1.68**	2.77^{ns}	1.67*

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

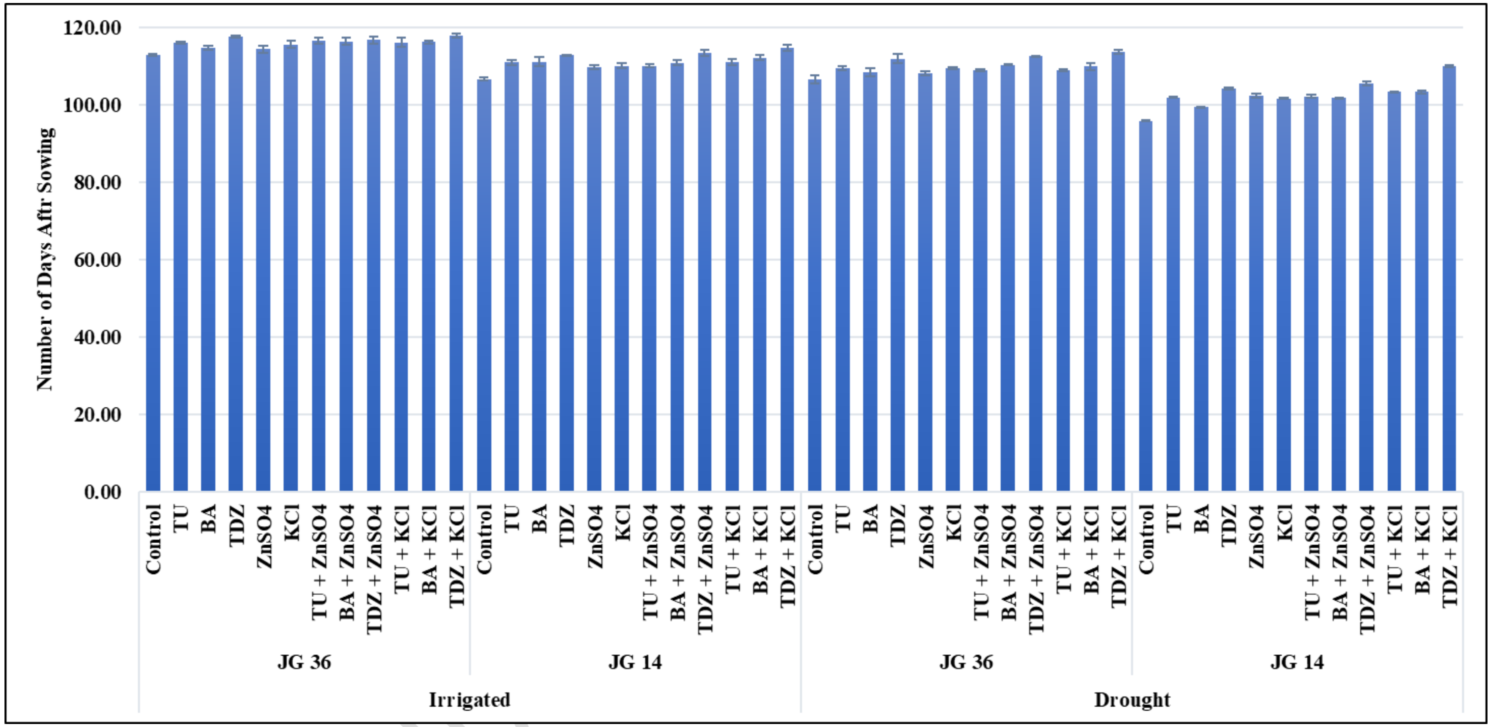
Table 4: Physiological maturity and Harvest maturity as affected by varied irrigation level, varieties, foliar spray of plant growth regulators and nutrients in Chickpea

Days to Physiological maturity				Days to Harvest maturity		
Treatments	**2021-2022**	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**
Main plot: Irrigation (D)						
D_1	102.47^{a}	101.89^{a}	102.18^{a}	112.38^{a}	114.65^{a}	113.51^{a}
D_2	98.44^{b}	95.21^{b}	96.83^{b}	106.98^{b}	105.51^{b}	106.25^{b}
SEm±	0.20	0.06	0.13	0.09	0.36	0.19
SD	0.29	0.08	0.18	0.14	0.51	0.26
Subplot: Varieties (V)						
V_1	106.42^{a}	104.24^{a}	105.33^{a}	113.19^{a}	112.61^{a}	112.90^{a}
V_2	94.50^{b}	92.85^{b}	96.67^{b}	106.17^{b}	107.55^{b}	106.86^{b}
SEm±	0.28	0.11	0.16	0.14	0.16	0.09
SD	0.39	0.16	0.23	0.21	0.23	0.13
Sub-sub plot: Treatments (T)						
T_1	95.45^{e}	97.09^{d}	96.27^{d}	104.40^{f}	106.50^{e}	105.45^{g}
T_2	99.98^{bcd}	98.64^{bcd}	99.31^{bc}	109.35^{cd}	109.80^{bcd}	109.57^{def}
T_3	97.78^{de}	97.54^{d}	97.66^{cd}	107.56^{e}	109.27^{cd}	108.41^{f}
T_4	103.04^{abc}	99.53^{bc}	101.29^{ab}	111.92^{b}	111.33^{bc}	111.62^{bc}
T_5	100.10^{bcd}	97.85^{cd}	98.97^{c}	108.53^{de}	108.76^{de}	108.65^{ef}
T_6	101.49^{abcd}	97.88^{cd}	99.69^{bc}	109.49^{cd}	108.90^{d}	109.19^{def}
T_7	100.45^{abcd}	98.21^{bcd}	99.33^{bc}	109.43^{cd}	109.47^{cd}	109.45^{def}
T_8	100.44^{abcd}	97.60^{d}	99.02^{c}	110.07^{c}	109.58^{cd}	109.82^{de}
T_9	103.77^{ab}	99.89^{ab}	101.83^{a}	112.16^{b}	111.97^{b}	112.07^{b}
T_{10}	99.42^{cde}	98.42^{bcd}	98.92^{c}	109.65^{cd}	110.03^{bcd}	109.84^{de}
T_{11}	99.13^{cde}	98.62^{bcd}	98.88^{c}	109.81^{cd}	110.97^{bcd}	110.39^{cd}
T_{12}	104.42^{a}	101.30^{a}	102.86^{a}	113.80^{a}	114.34^{a}	114.07^{a}
SEm±	0.89	0.36	0.44	0.30	0.48	0.29
SD	1.26	0.50	0.62	0.42	0.68	0.41

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

V_1 - JG 36 (Late maturity) **V_2 - JG 14 (Early maturity)**

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



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

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

In water deficit conditions, well-irrigated plots exhibited delayed maturity, while water-stressed plants showed an early onset of maturity, indicating resource reallocation as a survival strategy. Well-watered condition exhibited delayed phenological stages of the crop, while drought stress at flowering up to physiological maturity showed an early onset of maturity. Under water deficit stress condition, the plant may prioritize reproductive processes, including flowering and pod setting. This reallocation of resources toward reproductive parts is an escaping mechanism to which can expedite the onset of maturity. 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.

Comment [TK11]: I think it is no need to write

Thidiazuron (TDZ) significantly influenced phenological stages especially in combination with KCl, delayed both pod and seed formation, as well as physiological and harvest maturity. This delay in maturity could be attributed to the anti-senescence properties, inhibition of ethylene synthesis, simulation of cell division, and regulation of floral transition associated with TDZ. The combination of cytokinin analogs and nutrients proved to be a novel approach, offering insights for sustainable chickpea cultivation under water deficit conditions.

Comment [TK12]: I think it is more simple to write "TDZ @ 10 ppm + 1% KCl significantly influenced phenological stages i.e. delayed both pod and seed formation as well as physiological and harvest maturity.

Comment [TK13]: No need to write

TDZ @ 10 ppm + 1% KCl enhanced grain filling duration by 2.59 days over the untreated control. This shows the potential of TDZ to positively influence the seed development process by affecting histogenesis, seed filling, and seed maturation. 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, to provide recommendations for chickpea growers.

Comment [TK14]: No need to write

Comment [TK15]: showed

References

Ahmad, F., Gaur, P. M., & Croser, J. (2005). Chickpea (*Cicer arietinum* L.). *Genetic resources, chromosome engineering, and crop improvement-grain legumes*, *1*, 187-217.

Barnabás, B., Jager, K., & Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment*, *31*(1), 11-38.

Berger, J., Turner, N. C., & French, R. J. (2003, February). The role of phenology in adaptation of chickpea to drought. In *Solution for a better environment. Proceedings of the 11th Australian Agronomy Conference, Geelong, Victoria, Australia* (pp. 2-6).

Cho, L. H., Yoon, J., Tun, W., Baek, G., Peng, X., Hong, W. J., ... & An, G. (2022). Cytokinin increases vegetative growth period by suppressing florigen expression in rice and maize. *The Plant Journal*, *110*(6), 1619-1635.

Chowdhury, J. A., Karim, M. A., Khaliq, Q. A., Ahmed, A. U., & Khan, M. S. A. (2016). Effect of drought stress on gas exchange characteristics of four soybean genotypes. *Bangladesh Journal of Agricultural Research*, *41*(2), 195-205.

Currey, C. J., Lopez, R. G., Rapaka, V. K., Faust, J. E., & Runkle, E. S. (2013). Exogenous applications of benzyladenine and gibberellic acid inhibit lower-leaf senescence of geraniums during propagation. *Hort Science*, *48*(11), 1352-1357.

Devasirvatham, V., & Tan, D. K. (2018). Impact of high temperature and drought stresses on chickpea production. *Agronomy*, *8*(8), 145.

FAOSTAT. Annual Report. Statistics, food and agriculture organization of the United Nations. Food and Agriculture Organization of the United Nations; 2011.

Ferrante, A., Hunter, D. A., Hackett, W. P., & Reid, M. S. (2002). Thidiazuron—a potent inhibitor of leaf senescence in *Alstroemeria*. *Postharvest Biology and technology*, *25*(3), 333-338.

Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley & Sons; 1984

Hasanuzzaman, M., Bhuyan, M. B., Nahar, K., Hossain, M. S., Mahmud, J. A., Hossen, M. S., ... & Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*, *8*(3), 31.

Hu, J., Ren, B., Dong, S., Liu, P., Zhao, B., & Zhang, J. (2020). Comparative proteomic analysis reveals that exogenous 6-benzyladenine (6-BA) improves the defense system activity of waterlogged summer maize. *BMC plant biology*, *20*, 1-19.

Hussain, M., Mehboob, N., Naveed, M., Shehzadi, K., & Yasir, T. A. (2020). Optimizing boron seed coating level and boron-tolerant bacteria for improving yield and biofortification of chickpea. *Journal of Soil Science and Plant Nutrition*, *20*, 2471-2478.

Imsabai, W., Leethiti, P., Netlak, P., & van Doorn, W. G. (2013). Petal blackening and lack of bud opening in cut lotus flowers (*Nelumbo nucifera*): Role of adverse water relations. *Postharvest Biology and Technology*, *79*, 32-38.

Jose, L. P., André, A. S., Cristhian, L. F., et al. (2020). Increasing on fruit set and yield of ‘Monalisa’ and ‘Maxi Gala’ apple trees using plant growth regulators. *Journal of Experimental Agriculture International, 42*(5), 34–43.

Keerthana, S. M., Ramakrishnan, R. S., Nagre, S., Kumar, A., Sharma, R., Upadhyay, A., & Samaiya, R. K. (2024). Seed Germination and Seed Vigour Induction through Foliar Application of Plant Growth Regulators and Nutrients under Drought Stress in Chickpea (*Cicer arietinum* L.). *Archives of Current Research International*, *24*(1), 13-23.

Keerthana, S. M., Ramakrishnan, R. S., Pathak, N., Pawar, P. S., and Ghosh, D. (2022). Seed Rate and Sowing Method induced Variation in Phenology, Seed Yield and Seed Quality of Soybean [*Glycine max* (L.) Merill]. *Biological Forum – An International Journal*, 14(4a): 541-547.

Khan, H. R., McDonald, G. K., & Rengel, Z. (2003). Zn fertilization improves water use efficiency, grain yield and seed Zn content in chickpea. *Plant and Soil*, *249*, 389-400.

Li, F., Wu, Q., Liao, B., Yu, K., Huo, Y., Meng, L., ... & Li, Z. (2022). Thidiazuron promotes leaf abscission by regulating the crosstalk complexities between ethylene, auxin, and cytokinin in cotton. *International Journal of Molecular Sciences*, *23*(5), 2696.

Liu, X. S., Gu, W. R., Piao, L., Zhang, L. G., Zhou, Y., LI, C. F., ... & Wei, S. (2017). Effects of mixture of thidiazuron and ethephon on grain-filling characteristics and hormone regulation mechanism in spring maize. *Chinese Journal of Ecology*, *36*(12), 3526.

McVay, K., Burrows, M., Menalled, F., Jones, C., Wanner, K., & O'Neill, R. (2013). Montana cool-season pulse. *Production Guide*, 1-28.

Mok, M. C., Martin, R. C., & Mok, D. W. (2000). Cytokinins: biosynthesis metabolism and perception. *In Vitro Cellular & Developmental Biology-Plant*, *36*, 102-107.

Mutui, T. M., Mibus, H., & Serek, M. (2012). Effect of meta-topolin on leaf senescence and rooting in *Pelargonium × hortorum* cuttings. *Postharvest biology and technology*, *63*(1), 107-110.

Nisler, J. (2018). TDZ: mode of action, use and potential in agriculture. *Thidiazuron: from urea derivative to plant growth regulator*, 37-59.

Pasa, M. S., SILVA, C. P., Carra, B., Brighenti, A. F., SOUZA, A. L. K., & Petri, J. L. (2017). Thidiazuron (TDZ) increases fruit set and yield of 'Hosui'and 'Packham's Triumph'pear trees. *Anais da Academia Brasileira de Ciencias*, *89*, 3103-3110.

Patade, V. Y., Nikalje, G. C., & Srivastava, S. (2020). Role of thiourea in mitigating different environmental stresses in plants. *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives*, 467-482.

Pathak, G. C., Gupta, B., & Pandey, N. (2012). Improving reproductive efficiency of chickpea by foliar application of zinc. *Brazilian Journal of Plant Physiology*, *24*, 173-180.

Praba, M. L., Cairns, J. E., Babu, R. C., & Lafitte, H. R. (2009). Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. *Journal of Agronomy and Crop Science*, *195*(1), 30-46.

Pushpavalli, R. (2015). *Physiological and genetic deciphering of water, salinity and relative humidity stress in chickpea (Cicer arietinum L.)* (Doctoral dissertation, Bharathidasan University, Tiruchirappalli, Tamil Nadu).

Rivero, R. M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., & Blumwald, E. (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences*, *104*(49), 19631-19636.

Sachdeva, S., Bharadwaj, C., Patil, B. S., Pal, M., Roorkiwal, M., & Varshney, R. K. (2022). Agronomic performance of chickpea affected by drought stress at different growth stages. *Agronomy*, *12*(5), 995.

Sankhla, N., Mackay, W. A., & Davis, T. D. (2003, August). Effect of thidiazuron on senescence of flowers in cut inflorescences of *Lupinus densiflorus Benth*. In *VIII International Symposium on Postharvest Physiology of Ornamental Plants 669* (pp. 239-244).

Serraj, R., Krishnamurthy, L., Kashiwagi, J., Kumar, J., Chandra, S., & Crouch, J. H. (2004). Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Research*, *88*(2-3), 115-127.

Thalooth, A. T., Tawfik, M. M., & Mohamed, H. M. (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mungbean plants grown under water stress conditions. *World Journal of Agricultural Sciences*, *2*(1), 37-46.

Tiwari, R. K., & Kushwaha, H. S. (2020). Effect of Foliar Nutrition on Productivity and Profitability of Chickpea (*Cicer arietinum* L.) in Kymore Plateau of Madhya Pradesh. *International Journal of Current Microbiology and Applied Sciences*, *9*(09), 1333-1338.

Toscano, S., Trivellini, A., Ferrante, A., & Romano, D. (2018). Physiological mechanisms for delaying the leaf yellowing of potted geranium plants. *Scientia Horticulturae*, *242*, 146-154.

Turner, N. C. (2004). Agronomic options for improving rainfall-use efficiency of crops in dryland farming systems. *Journal of Experimental Botany*, *55*(407), 2413-2425.

Van Doorn, W. G., Perik, R. R., Abadie, P., & Harkema, H. (2011). A treatment to improve the vase life of cut tulips: Effects on tepal senescence, tepal abscission, leaf yellowing and stem elongation. *Postharvest Biology and Technology*, *61*(1), 56-63.

Vineeth, T. V., Kumar, P., & Krishna, G. K. (2016). Bioregulators protected photosynthetic machinery by inducing expression of photorespiratory genes under water stress in chickpea. *Photosynthetica*, *54*, 234-242.