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Research article

Mitigation of water limitation effects on flower traits, fruit development and yield of chili pepper (*Capsicum annuum* L.) by brassinosteroid mimic

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Abstract

Importance of the work: 7,8-Dihydro-8α-20-hydroxyecdysone (DHECD) is a brassinosteroid mimic, that could enhance the resilience and sustainability of chili pepper cultivation, which frequently has water shortage problems, particularly in Northeast Thailand.

Objectives: to investigate the impact of DHECD on chili pepper traits, flower anatomy, fruit development and yield under well-irrigated and water-limited conditions.

Materials & Methods: Four treatments (well irrigated with and without DHECD and water limitation with and without DHECD) were applied during the floral induction stage of development. Under water-limited conditions (50% pot water capacity for 5 d), 0.1 µM DHECD was applied to plants aged 60 d during the floral induction stage of reproductive development.

Results: DHECD enhanced floral length, influencing flower anatomy by promoting the tissue layer that merged with the ovary wall to form the pericarp, as well as facilitating tapetum layer formation under water-limited conditions. DHECD alleviated the delays in flower, microspore and fruit development caused by the water limitation. Furthermore, DHECD ensured regular fruit growth, thick pericarp layer development, increased pollen germination, a high fruit set percentage and reduced fruit drop percentage.

Main finding: DHECD application during the floral induction stage strategically enhanced chili pepper resilience to water limitation, presenting a valuable approach for sustainable production in water-limited environments.

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Introduction

Low water availability is a major limitation for plant production worldwide, with drought impacting the growth and development of crop plants. The effects of water limitation on a particular species depend on various factors, including drought severity and plant developmental stage (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). During floral induction, drought causes abnormal flower morphology, delayed flowering and complete inhibition of flowering leading to yield loss (Barnabás et al., 2008). Water shortage impacts all reproductive stages including sporogenesis and gametogenesis, pollination and embryo development (Alqudah et al., 2011). Saini and Westgate (1999) suggested that the process of meiosis during sporogenesis was the most stress-sensitive stage in plant reproduction, with drought during this period resulting in gamete sterility and pollination failure. Drought affected pollen and tapetum development, leading to a reduction in wheat grain number (Dong et al., 2017). Jin et al. (2013) noted that rice microspore tetrads failed to separate into single microspores under water deficit and the tapetum vacuolated and collapsed. Drought negatively impacts many male-expressing genes during both the meiotic and post-meiotic stages. For example, Su et al. (2013) reported floral defects including malformed anthers and low pollen viability in *Arabidopsis* under water shortage. Their transcriptomics study suggested that the stamen was more sensitive to water stress than the pistil. A study in chickpeas (*Cicer arietinum* L.) revealed that water deficiency caused smaller flowers and impaired the function of the pistils for pollen tube growth (Fang et al., 2010). The effects of drought on reproductive processes have also been reported. Drought conditions inhibited mitotic cell division and cell growth, resulting in reduced plant growth and yield (Aslam et al., 2015), while other studies observed that water deficit caused delayed floral formation (Craufurd et al., 1993; Winkel et al., 1997). In addition, evidence revealed abnormal ovary development caused by water stress in maize (Boyer and Westgate, 2004). Water deficit in olives during early fruit development led to low fruit fresh weight and volume, with a decreased area of the fruit mesocarp (Rapoport et al., 2004).

Chili pepper (*Capsicum annuum* L. var. *frutescens* (L.) Kuntze) is an important household culinary ingredient, providing a hot, spicy taste. In addition, it is used as a component in pharmacy products due to the capsaicinoids in the fruit (Suzuki and Iwai, 1984). Water limitation affects chili growth and also impacts fruit yield and capsaicin production (Phimchan et al., 2012, Zamljen et al., 2020). In Northeast Thailand, water scarcity during the reproductive growth phases of chili peppers is a major obstacle for cultivators, as inadequate rainfall and limited irrigation resources contribute to water-limited stress during this period. Insufficient water availability negatively affects flower development, fruit set and yield. Consequently, it is important to investigate effective approaches to alleviate the negative impacts of water shortage conditions to ensure sustainable and resilient chili production.

Brassinosteroids (BRs), a family of natural steroid hormones, play an important role in plant morphogenesis and development through cell division and cell elongation (Clouse and Sasse, 1998). Several studies have shown that BRs regulate pollen tube growth (Vogler et al., 2014; Tepkaew et al., 2022; Wang et al., 2023). Furthermore, BRs are responsible for plant defense mechanisms against various abiotic stresses, including high temperature, salt and drought (Krishna, 2003; Fariduddin et al., 2014). 7,8-Dihydro-8α-20-hydroxyecdysone (DHECD) is a BR mimic that has a chemical structure similar to the natural BRs (Zullo et al., 2003). It can be generated using catalytic hydrogenation of 20-hydroxyecdysone extracted from the bark of *Vitex glabrata* (Suksamrarn et al., 2002). DHECD effectively maintained chili plant growth under drought-stress conditions during vegetative growth by increasing the net photosynthetic rate, thereby maintaining chlorophyll fluorescence and membrane integrity (Khamsuk et al., 2018). Furthermore, DHECD application increased pollen germination, seed set and antioxidant enzyme activities under heat-stress conditions (Thussagunpanit et al., 2013; Sonjaroon et al., 2016). It was hypothesized that the DHECD application may be an effective solution to alleviate the water shortage drawbacks during chili pepper cultivation. Therefore, the current study investigated the effectiveness of DHECD on the reproductive development and yield of chili peppers under well-irrigated and water-limited conditions.

Materials and Methods

Plant growth condition and maintenance

Seeds of chili pepper (*Capsicum annuum* L.) cv. TVRC 758 were obtained from the Tropical Vegetable Research Center, Research and Development Institute, Kasetsart University, Thailand. Initially, the seeds were sown in a soil and sand mixture; after reaching age 30 d, the seedlings were transplanted into pots containing a mixture of loam soil, rice husk ash and

animal manure (in a 2:2:1 volume ratio). This transplantation took place within a greenhouse at the Department of Botany, Kasetsart University, Thailand (13°50'41.0" N 100°34'15.1" E). Throughout the experiment, the seedlings were cultivated under controlled conditions with average temperatures of 31.98°C and 27.26°C for 12 hr day and 12 hr night, with midday average photosynthetic photon flux density of 658.51 μmol/m^2 s and relative humidity of 60–75%. Weather data were recorded using a Watchdog 1450 data logger. Atter 60 d after transplantation, the plants were subjected to four different treatments. The first two treatments comprised well-irrigated with and without DHECD applications. The well-irrigated treatment without DHECD served as the control (C), while the well-irrigated with DHECD application was labeled as D. The well-irrigated treatment maintained the pot water capacity at 100%. In contrast, the remaining two treatments were water-limited conditions: one with the application of DHECD (SD) and the other without the application of DHECD (SC). Under these water-limited conditions, the plants experienced mild water limitation at 50% pot water capacity for a duration of 5 d. The DHECD application was performed by spraying the DHECD solution at 0.1 μM concentration, where the dosage was based on studies indicating that this concentration effectively enhanced plant tolerance (Thussagunpanit et al., 2013), at 10 mL per plant, once on the 1st day of water limitation. Each treatment consisted of five plants as replications.

Measurement of floral length

In the experimental setup, five individual flowers per replication were identified and tagged with string. These tagged flowers were selected for continuous monitoring of floral length. Floral length measurements were taken at 0 d, 2 d, 4 d and 7 d after treatment.

Anatomical observation of flower and pollen development

Floral buds (1 mm diameter, 10 buds per plant) were tagged at the beginning of the experiment and then collected at 1 d, 4 d, 7 d and 11 d after treatment application and immediately fixed in a 50% formalin-acetic acid-alcohol (FAA) solution. Permanent slides were prepared for microscopic observation using standard microtechnique procedures, as described by Johansen (1940). The samples were sectioned to a thickness of 10−15 μm using a rotary microtome (Leica Biosystems; Germany) and stained with Safranin O, followed by Fast Green. Observation of the samples for flowers and anthers was conducted using a bright-field microscope (Olympus; Japan).

Estimation of in vitro pollen germination

Pollen grains from the anthers of full-bloom tagged flowers were collected during 0800–1100 hours. For each treatment, 10 mg of pollen grains were cultured in a liquid medium containing 10% sucrose, 0.1 g/L H_3BO_3, 0.3 g/L $CaNO_3$, 0.2 g/L $MgSO_4$ and 0.1 g/L KNO_3 (Brewbaker and Kwack, 1963). The pollen grains were cultured using the hanging drop technique (Hewitt et al., 1985) and left to germinate in the dark at 25°C for 24 hr. Pollen germination was quantified using a bright-field microscope (Olympus; Japan), recording both the total number of pollen grains and the number of germinated pollen. Pollen grains were considered germinated when the pollen tube length was at least twice the pollen grain diameter. Data were presented as a percentage of pollen germination using the formula: Percentage of pollen germination = (Number of germinated pollen / Total number of pollen grains) × 100.

Anatomical observation of fruit development

Full-bloom flowers were tagged at the onset of the experiment for each treatment and collected for anatomical study at 1 d, 4 d, 7 d and 11 d after pollination and immediately fixed in 50% FAA solution. Permanent slides for microscopic observation were prepared using standard microtechnique procedures (Johansen, 1940). The samples were cross-sectioned to 10−15 μm thickness using a rotary microtome (Leica Biosystems; Germany), stained with Safranin O followed by Fast Green and observed under a bright-field microscope (Olympus, Japan).

Determination of fruit set, fruit drop, fruit size and yield

On the day of pollination, the number of full-bloom flowers was counted and tagged for the continual assessment of the fruit set. After 2 d of pollination, the fruits from these tagged flowers were recorded as the initial fruit count. The percentage of fruit set was determined using the formula: Percentage of fruit set = (Number of initial fruits / Number of blooming flowers) × 100. The percentage of fruit drop was calculated based on the remaining tagged fruits at harvest using the formula: Percentage of fruit drop = (Number of remaining fruits / Number of initial tagged fruits) × 100.

Furthermore, the fresh weight per fruit and total yield were measured at harvest time. The fruit length was measured at 0 d, 2 d, 4 d and 7 d after pollination to monitor fruit growth.

Statistical analyses

All experiments were performed following a completely randomized design with five replicates. Results were presented as mean ± SD Data were subjected to analysis of variance using SPSS statistical software (version 20.0; SPSS Inc.; Chicago, IL, USA). Significant differences among means were determined using Tukey's honest significant difference test at $p < 0.05$.

Results and Discussion

Measurement of floral length

Floral length responded significantly to the DHECD application. Under well-irrigated conditions, plants treated with DHECD (D treatment) exhibited greater flower length than those without DHECD (C treatment), particularly during the early stage of flower development on day 2 (Fig. 1). On the other hand, under the water-limited conditions, plants receiving DHECD (SD treatment) showed greater flower length than untreated plants (SC treatment) during the later stage of flower development on day 7 (Fig. 1). These results indicated that DHECD positively affected floral length under both irrigation regimes, with time-dependent effects. In well-irrigated conditions, increased flower length was observed during early development, while in water-limited conditions, the impact was prominent during the late stages. This result provides valuable information for improving floral length responses to various environmental conditions and concurred with Tepkaew et al. (2022), who demonstrated that DHECD application increased inflorescence size and accelerated inflorescence development in mango during the early stages. DHECD reportedly increases cell elongation by regulating BR-related genes (Thussagunpanit et al., 2017), which work synergistically with auxin to stimulate cell elongation (Cohen and Meudt, 1983). Notably, following prolonged water limitation, as observed on day 7 (Fig. 1), the floral length in SD plants was approximately twice that in SC plants, indicating the potential for DHECD to mitigate stress conditions, as described by Sonjaroon et al. (2016), who suggested that DHECD reduced the impact of oxidative free radicals and enhanced the antioxidative enzyme system, thereby mitigating abiotic stress across plant species.

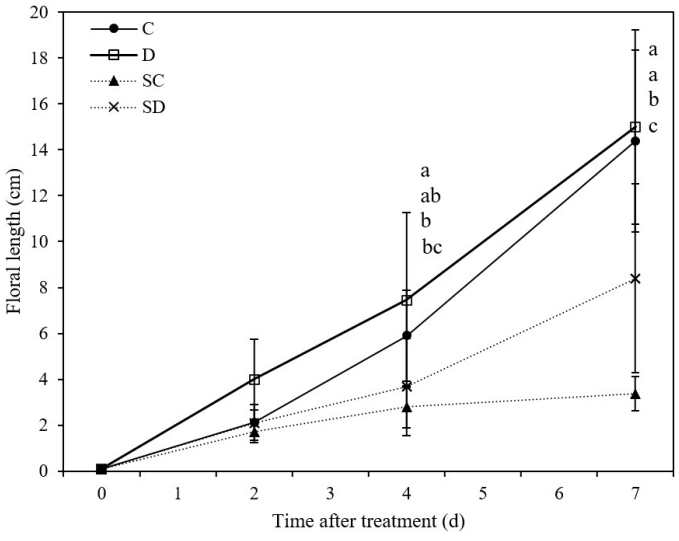

Fig. 1 Floral length of chili pepper at 0–7 d after treatment, where C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD. Data are presented as mean ± SD ($n = 5$). Different lowercase letters at each time point indicate significant differences ($p < 0.05$).

Anatomical observation of flower and pollen development

The anatomy of the floral tissues was examined to assess the reproductive damage caused by water limitation. Initially, on days 1, 4 and 7 of data collection during flower development, there were no noticeable differences among treatments. All flowers in each treatment had a regular floral composition (Figs. 2A–2L). All treatments displayed complete floral structures, including sepals, petals, anthers and ovaries (Figs. 2I–2L). In particular, water limitation did not interfere with the formation of floral organs (Figs. 2K, 2L), as observed on day 7 of data collection, indicating that despite the water limitations in this study, the flowers still had the potential to retain their fertility and remain capable of fruit production. By day 11 of data collection, distinctions became apparent between the well-irrigated and water-limited treatments. There was a noticeable change in the tissue layer that merged with the ovary wall to form the pericarp during the late stages of flower development (Figs. 2M–2P). In the well-irrigated treatments, the pericarp layer within the flowers appeared thick and dense (Figs. 2M, 2N), whereas it was not present in the SC treatment (Fig. 2O), suggesting that water limitation negatively influenced and delayed pericarp formation. However, when DHECD was applied to mitigate the effects of water limitation in the SD treatment, evidence of a pericarp layer was observed (Fig. 2P). These findings suggested that DHECD plays a role in pericarp layer formation in flowers under water-limited conditions.

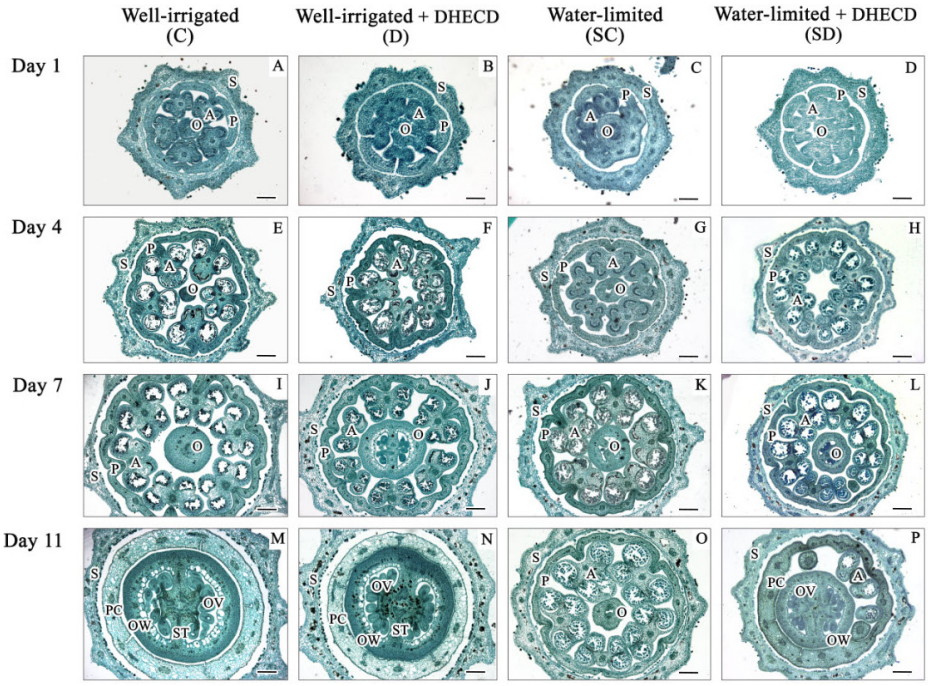

Fig. 2 Anatomical observation of chili pepper flowers at days 1, 4, 7 and 11 after treatment application, where C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD, A = anther, S = Sepal, ST = septum, O = ovary, OV = ovule, OW = ovary wall, P = petal, PC = pericarp. Scale bars = 200 μm.

Primarily, water limitation impacted the development of microsporocytes rather than affecting other floral organs. Based on the current results, plants subjected to water-limited conditions had delayed another development, ultimately resulting in the production of abnormal pollen grains. The effects of this delayed development due to water limitation became evident as early as the day 4 after treatment (Fig. 3G). In the SC plants, the anthers still contained sporogenous cells with no evidence of anther sac formation (Fig. 3G), unlike in the other treatments, where anther sacs, tetrads of microspores and a tapetum layer were present (Figs. 3E, 3F and 3H). On day 7 after treatment, the anther wall had reached full development across all treatments (Figs. 3I–3L). However, a noticeable difference was observed; the anther wall of both C and D treatments (Figs. 3I–3J) was visibly thicker than the anther wall in the SC and SD treatments (Figs. 3K–3L). The tapetum layer was present in the S and D treatments (Figs. 3I–3J) and the SD treatment (Fig. 3L) but absent in the SC treatment (Fig. 3K). Furthermore, the microspores of both the C and D treatments developed into mature pollen grains (Figs. 3I–3J), whereas in the SC treatment, they remained in the tetrad stage of development (Figs. 3K). This observation suggested that water limitation might have had a negative effect on the formation of the anther wall and tapetum layer and delayed microspore development. Following the application of DHECD, there was evidence that the endothecium layer of D (Fig. 3J) was thicker than for the C treatment (Fig. 3I), which was comparable to the SD treatment (Fig. 3L) having a thicker endothecium than the SC treatment (Fig. 3K). Furthermore, under water-limited conditions, there was a noticeable difference in the tapetum layer, with its presence in the SD treatments and absence in the SC treatments (Fig. 3K). By day 11, the tapetum layer had degraded in all treatments (Figs. 3M, 3N and 3P). However, in the SC treatment (Fig. 3O), certain microspores persisted in the tetrad stage, the tapetum was still present and pollen grains were not fully stained with Fast Green, in contrast to the other treatments (Figs. 3M, 3N and 3P).

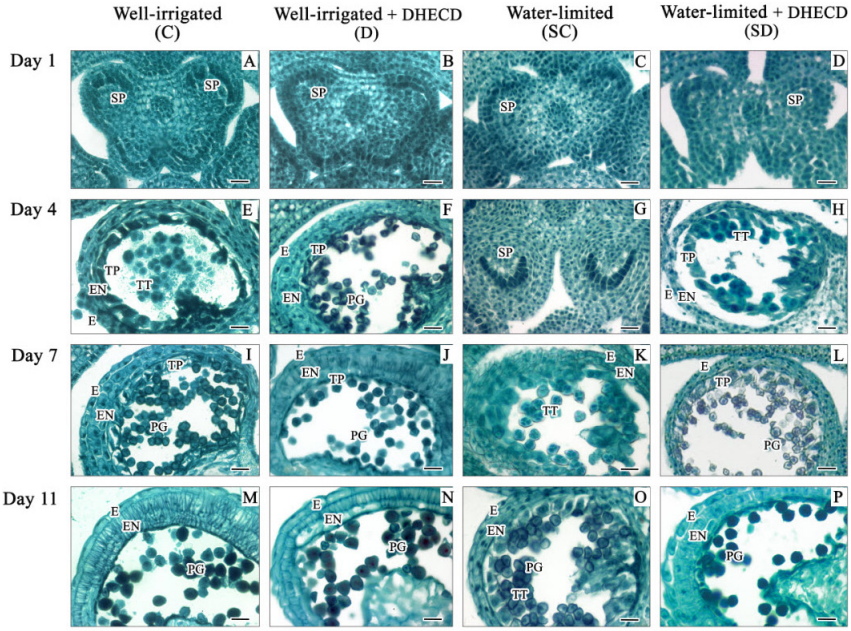

Fig. 3 Anatomical observation of chili pepper anthers at days 1, 4, 7 and 11 after treatment application, where C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD, E = epidermis, EN = endothecium, PG = pollen grain, SP = sporogenous cell, TP = tapetum, TT = tetrads. Scale bars = 20 μm.

Salter and Goode (1967) reported that water stress delays microspore development, reducing pollen viability and ultimately affecting crop yield. Their finding emphasize that different growth stages vary in sensitivity to water deficits, with flowering being particularly venerable due to high water demand. The current findings aligned with Dong et al. (2017) who reported an aberrant and degrading tapetum layer in wheat anthers under drought stress. During pollen development, BRs initiate and maintain the tapetum layer (Li and He, 2020), playing a regulatory role in the expression of key genes and transcription factors involved in anther and pollen development. BRs regulate the transcription factors *BES1* (*bri1-EMS-Suppressor 1*) and *BIM1* (*BES1-interacting Myc-like 1*), which are known to influence anther development and pollen formation (Li, 2010; Ye et al., 2010). Consequently, in the current study, the application of DHECD alleviated reproductive damage caused by water limitation. The microspores of the SD plants developed into functional mature pollen within a timeframe similar to the well-irrigated conditions and restored the intact tapetum layer, finally promoting microspore development.

Effects of Estimation of in vitro pollen germination

Water limitation significantly reduced the percentage of *in vitro* pollen germination. In the SC treatment, the percentage of pollen germination was 12.3% lower than in the C treatments. The application of DHECD showed the potential to improve the percentage of pollen germination and minimize the effects of water limitation. Under well-irrigated conditions, the D plants had a 1.8% increase in pollen germination compared to the C plants, while under water-limited conditions, the increase was 5.1% for the SD treatment compared to the SC treatment. However, these differences were not significant (Fig. 4).

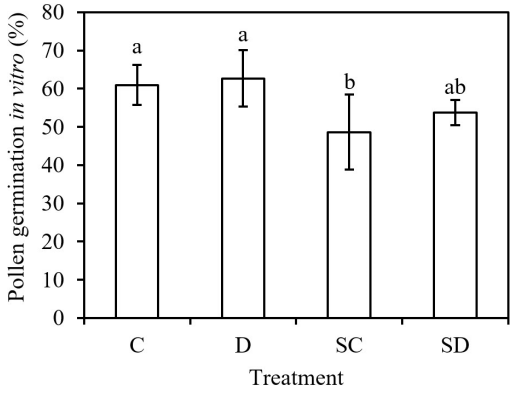

Fig. 4 Percentage of *in vitro* pollen germination after treatment application, where C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD. Data are presented as mean ± SD (n = 5); C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD. Different lowercase letters above bars indicate significant differences ($p < 0.05$) among means.

Pollen germination reflects the functionality of mature pollen produced under different treatment conditions. Water limitation impacted pollen by reducing carbohydrate mobilization, thereby inhibiting pollen tube growth (Hu et al., 2019). BRs may play a role in male fertility, including anther and pollen development in *Arabidopsis* (Ye et al., 2010). Improved pollen germination resulting from DHECD application concurred with the findings of Tepkaew et al. (2022) who had studied pollen-pistil interaction in mango.

Anatomical observation of fruit development

During the initial phase of development, specifically on day 1 after treatment, there were no significant differences observed in the anatomy of the fruit tissue (Figs. 5A–5D). On day 4 after treatment, it became more evident that the C and SD treatments were developing more rapidly than the D and SC treatments, based on the fruit in the C and SD treatments reaching the locular formation stage, where the locules are visible (Figs. 5E and 5H). In contrast, the remaining two treatments did not show any signs of locule development (Figs. 5F and 5G). Subsequently, on day 7 after treatment, there was a notable disparity between the well-irrigated and water-limited conditions. The fruit development in the SC treatment was delayed, with the fruit only reaching the stage of locule formation and showing no signs of ovules, septum or seeds (Fig. 5K), whereas in the other treatments, the placenta, funiculus and seeds were detectable (Figs. 5I, 5J and 5L). DHECD application demonstrated the potential to minimize the effects of water limitation and enable regular fruit growth. In the SD treatment, the fruit had regular growth within the same timeframe as the well-irrigated treatments (C, D). Nevertheless, water limitation still negatively impacted the SD treatment, resulting in a thinner endocarp layer with smaller giant cells (Fig. 5L) compared to the well-irrigated treatments (Figs. 5I, 5J). However, the mesocarp and exocarp remained unchanged, with no noticeable differences observed. This result concurred with observations in olive fruit, where drought significantly altered the timing of endocarp growth (Rapoport et al., 2004). In the current study, on day 11 after treatment, the fruit and seeds in the C, S and SD treatments (Fig. 5M, 5N and 5P) were enlarged, while seed formation in the SC treatment was delayed (Fig. 5O). Water limitation delayed fruit development in a similar pattern to flower development. Drought altered assimilation partitioning and constrained water and dry mass accumulation in the fruit, resulting in defects in fruit development (Lopez et al., 2006; Dietz et al., 2021). Another report indicated that BRs induce the expression of cell cycle-related genes (*CycA, CycB, CycD3;1, CycD3;2* and *CDKB*), thereby regulating early-stage fruit development (Fu et al., 2008). Therefore, the current results highlighted the efficacy of DHECD application in mitigating the adverse effects of water limitation on fruit development and ensuring consistent fruit development within the expected period.

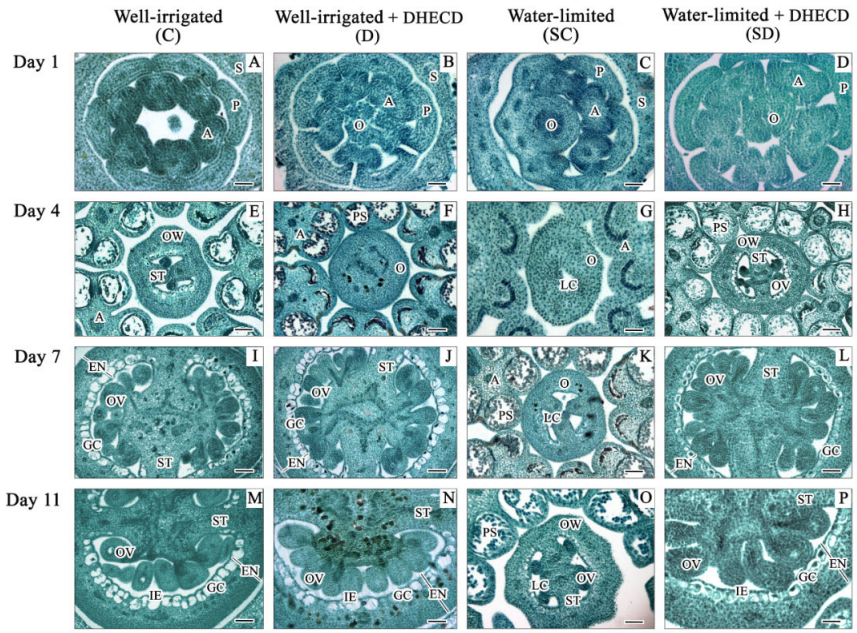

Fig. 5 Anatomical observations of chili pepper fruit at days 1, 4, 7 and 11 after treatment application, where A = anther, EN = endocarp, GC = giant cells, IE = internal epidermis, LC = locule, MS = mesocarp, O = ovary, OV = ovule, OW = ovary wall, P = petal, PS = pollen sac, S = sepal, ST = septum. Scale bars = 50 μm.

Determination of fruit set, fruit drop, fruit size and yield

The fruit set was reduced when chili pepper plants were subjected to water-limited conditions during the reproductive stage. The fruit set of the SC treatment was only 37.72%, which was 15.57% lower than that of the C treatment. However, after spraying with DHECD (SD), there was a notable increase in the fruit set of 14.06% compared to the SC treatment (Fig. 6A). Additionally, under well-irrigated conditions, DHECD increased the fruit set by 6.26% compared to the control, although this difference was not significant. The results of the current study aligned with the findings from Tepkaew et al. (2022), who observed that the application of DHECD positively influenced fruit set in mango under normal conditions. Their study revealed that DHECD improved pollen fertility, contributing to more successful fertilization and ultimately resulting in an increased fruit set. Similarly, Jangid and Dwivedi (2017) reported consistent results under drought conditions, demonstrating that the application of 24-epibrassiolide (EBL), a type of BR, enhanced tomato fruit set by 18.45%. These consistent results from various research experiments support the ability of BR-related compounds, such as DHECD and EBL, to enhance fruit set under varied environmental conditions.

Water limitation had a significant impact on the percentage of fruit drop determined at harvest time. Fruit drop was as high as 62.27% in the SC treatments, compared to only 48.21%, 46.70% and 40.43% in the SD, C and D treatments, respectively (Fig. 6B). Based on these results, DHECD application effectively reduced fruit drop, producing results comparable to well-irrigated conditions. In the well-irrigated treatments (C and D), fruit length, fruit weight and total yield were greater than for the water-limited treatments (SC and SD), as shown in Figs. 6C, 6D and 7. Based on a study of custard apples, drought during fruit development reduced average fruit size by 11% (George and Nissen, 2002). Although the application of DHECD tended to enhance fruit weight, fruit length and total yield under water-limited conditions, the increase was not significant (Figs. 6C, 6D and 7). However, the fruit length in the SD treatment was significantly longer than in the SC treatment after only day 4 of fruit development (Fig. 7). BRs have been reported to enhance fruit size and yield under optimal growth conditions (Serna et al., 2012). Furthermore, in a study by Khamsuk et al. (2018), the application of DHECD produced a notable 55% increase in chili pepper fruit weight under drought conditions. The disparity of the current results with these other reports could be attributed to several factors. First, the severity of water limitation could have influenced the variations in experimental results. It is possible that more severe water limitations than those applied in the current study could lead to statistically different results.

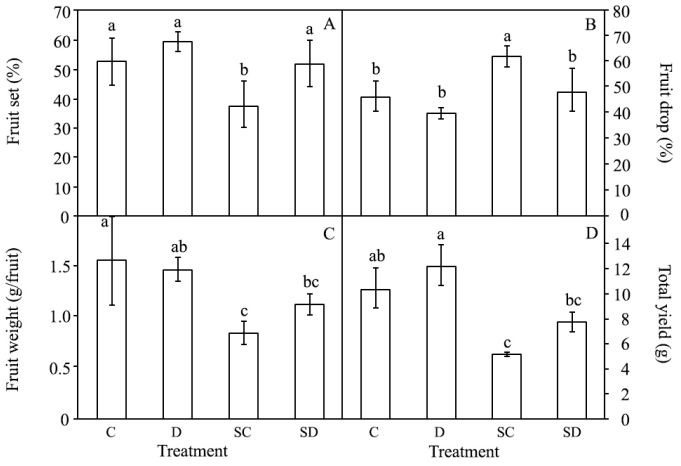

Fig. 6 Effects of water limitation and 7,8-dihydro-8α-20-hydroxyecdysone (DHECD) on chili pepper fruit traits: (A) fruit set; (B) fruit drop; (C) fruit weight; (D); total yield. Data are presented as mean ± SD (n = 5); treatments C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD. Different lowercase letters above bars indicate significant differences ($p < 0.05$).

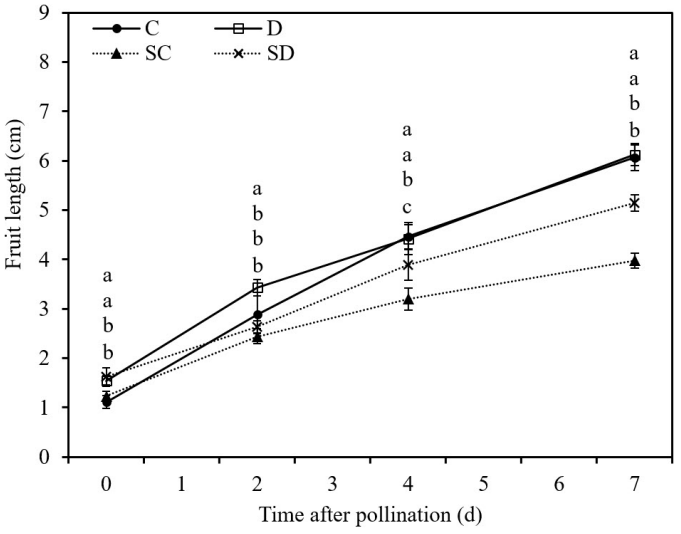

Fig. 7 Fruit length of chili pepper at days 0–7 after treatments, where C = well-irrigated without 7,8-dihydro-8α-20-hydroxyecdysone (DHECD), D = well-irrigated with DHECD, SC = water-limited without DHECD, SD = water-limited with DHECD. Data are presented as mean ± SD (n = 5). Different lowercase letters above bars indicate significant differences ($p < 0.05$).

The observed effects of DHECD under water-limited conditions were likely driven by the well-documented physiological and molecular roles of BRs in plant development and stress tolerance. For example, BRs enhance floral length by regulating cell division and elongation through genes responsible for cell wall loosening and expansion, such as expansins (Zhu et al., 2023). Additionally, BRs enhance gibberellin (GA) biosynthesis, stimulating further cell expansion, leading to increased floral organ elongation (Wan et al., 2024). Under water stress, BRs help maintain cell turgor pressure, sustaining normal floral organ growth. In terms of ovary wall tissue development and pericarp thickening, BRs regulate cell differentiation and expansion by modulating transcription factors, such as BZR1 and BES1, which activate genes involved in cell wall biosynthesis (Li et al., 2025). DHECD may interact with cytokinins to promote cell division in the ovary wall, contributing to thicker pericarp formation. Additionally, DHECD enhances photosynthate allocation to developing fruits, ensuring adequate carbohydrate supply for pericarp development. Furthermore, BRs play a vital role in tapetum layer formation and pollen germination that are essential for successful fertilization. By enhancing tapetal cell differentiation and regulating genes, such as AMS, MS1 and TDF1, BRs promote the biosynthesis of lipids and sporopollenin, ensuring proper pollen wall development (Zakharova et al., 2022). Furthermore, DHECD improves pollen germination and tube growth by modulating Ca^{2+} flux, thereby enhancing fertilization efficiency under both optimal and stress conditions (Tepkaew et al., 2022). Regarding flower, microspore and fruit development, BRs reduce delays in development by accelerating flowering and reproductive organ differentiation through activation of FLOWERING LOCUS D (FLD) and interactions with GA to regulate flowering time genes (Izawa, 2021). In addition, BRs prevent the accumulation of abscisic acid, which can delay flowering and fruit development, while stimulating carbohydrate metabolism and sugar transport to reproductive tissues, ensuring steady growth under water-limited conditions (Muhammad Aslam et al., 2022). Lastly, BRs and DHECD contribute to a higher percentage of fruit set and reduced fruit drop by modulating hormonal signaling and nutrient allocation; BRs reduce abscission zone sensitivity to ethylene by downregulating genes involved in fruit drop and reinforcing cell wall integrity, which enhances fruit retention (Ma et al., 2021). They also improve source-to-sink carbohydrate allocation, ensuring developing fruits receive sufficient nutrients (Xu et al., 2015; Liu et al., 2022). Furthermore, BRs increase auxin levels in developing fruits, strengthening fruit attachment and preventing premature fruit drop (Ramos et al., 2019). Together, these mechanisms help sustain reproductive success and ensure a stable yield, even under water-limited conditions.

Conclusions

DHECD had significant positive effects on floral and reproductive parameters in chili pepper plants under water-limited conditions. DHECD enhanced floral length, promoted regular flower and fruit development and mitigated the adverse impact of water limitations on fruit set and fruit drop. The anatomical observations revealed that DHECD played a crucial role in maintaining the development of the ovary wall into the pericarp and promoting the formation of the tapetum layer, hence facilitating timely pollen development. Furthermore, the DHECD application contributed to improving pollen germination, emphasizing its potential to alleviate the negative effects of water limitation on male fertility. Therefore, the application of DHECD presents an effective solution to mitigate the unfavorable effects of water limitation and promote consistent reproductive success. Further research could explore the optimal application rate and timing, fostering a deeper understanding of DHECD's broader implications for diverse crops and environmental conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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