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Influence of Irrigation Levels on the Phenology and Physiological Responses of Apple (*Malus domestica* Borkh.) Genotypes under Tropical Highland Conditions

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Abstract

The study examines the effects of different irrigation levels on the phenology and physiological responses of apple trees (*Malus domestica* Borkh) cultivated in tropical highland environments. Tropical highlands pose unique challenges to temperate crops like apple due to fluctuating temperature and water availability. Instead of optimizing irrigation per se, the study evaluates the influence of four irrigation treatments—70%, 90%, 110%, and 130% of crop evapotranspiration (*ETc*)—on key growth and physiological parameters using a split-plot design with two apple cultivars (Anna and Dorsett Golden). *ETc* was estimated using local reference evapotranspiration (*ETo*) adjusted with crop-specific coefficients (*Kc*). Phenological data were recorded across developmental stages: bud break, flowering, fruit set, and harvest. Physiological measurements included chlorophyll index, relative water content, net photosynthesis, stomatal conductance, transpiration, internal CO_2 concentration, leaf temperature, and instantaneous carboxylation efficiency. Biochemical analyses included reducing sugars, total soluble sugars, non-reducing sugars, and protein contents in leaves. Findings indicate that moderate irrigation (around 90–110% *ETc*) supported optimal phenological development and physiological efficiency, contributing to improved fruit yield and quality. In contrast, deficit irrigation delayed development and suppressed physiological performance, while excessive irrigation caused root zone saturation and health issues. These results highlight the need for balanced irrigation management to support apple cultivation in tropical highlands.

Keywords: *Malus domestica*, Phenology, Physiological Responses, Tropical Highlands, Crop Evapotranspiration (*ETc*), Net Photosynthesis, Irrigation Levels.

1. Introduction

Apple (*Malus domestica* Borkh) cultivation in tropical highlands poses significant agronomic challenges due to a mismatch between the crop's temperate climatic requirements and the unique environmental conditions of these regions (Einhorn & Caspari, 2004). Traditionally, apple varieties thrive in temperate climates characterized by cooler temperatures and distinct seasonal changes, which support dormancy, flowering synchronization, and fruit development (Mills et al., 1997; Faust & Soost, 1995). In contrast, tropical highlands—despite having relatively moderate altitudinal climates—often lack clear seasonal transitions and experience variable water availability and elevated temperatures (Ashebir et al., 2010; Scholefield et al., 2013). These factors can adversely affect phenological development, physiological efficiency, and ultimately fruit yield and quality.

Among the most critical factors influencing apple production under such conditions is water availability (Girona et al., 2010 a&b). Effective irrigation management is essential to mitigate climatic stress and ensure optimal tree performance (Hernandez et al., 2009; Felmann, 1996). In temperate regions, previous studies have shown that different irrigation regimes directly influence developmental stages such as bud break, flowering, fruit set, and harvest timing, along with physiological processes like stomatal regulation and photosynthesis (Williams et al., 1997; Jackson, 2008). However, limited research has investigated these relationships under tropical highland conditions, where conventional temperate irrigation strategies may be suboptimal due to the unique climate–crop interactions (Ntshidi et al., 2023).

This study addresses this knowledge gap by evaluating the impact of four irrigation levels—70%, 90%, 110%, and 130% of crop evapotranspiration (*ETc*)—on the phenological development, physiological functioning, and biochemical status of apple trees grown under tropical highland conditions. The irrigation treatments were designed based on ETc values calculated from local reference evapotranspiration (*ETo*) adjusted with crop coefficients (*Kc*) specific to apple developmental stages (Fallahi et al., 2010). The experiment was conducted using a split-plot design, with two cultivars—Anna and Dorsett Golden—as subplots, to assess varietal response variation under controlled irrigation scenarios.

The two cultivars, ‘Anna’ and ‘Dorsett Golden’, were selected for this study due to their low chilling requirements, making them particularly well-suited to mild winter tropical conditions such as those found in areas like Debre Birhan. Both cultivars are popularly cultivated in the study region, reflecting their adaptability and local importance. Additionally, Dorsett Golden is self-fertile and serves as an effective pollen donor to Anna, with good synchronization in pollination timing, which promotes successful fruit set. This selection allows for the evaluation of varietal responses under different irrigation regimes in tropical highland environments.

Key physiological parameters assessed include relative water content, chlorophyll index, net photosynthesis, stomatal conductance, transpiration rate, internal CO_2 concentration, and instantaneous carboxylation efficiency. In addition, biochemical characteristics such as reducing sugars, total soluble sugars, non-reducing sugars, and protein content in leaves were evaluated to provide a holistic understanding of tree health and performance. The findings of this study aim to provide evidence-based insights into how irrigation level affects apple tree development and function in tropical highland environments. While the study does not optimize irrigation in the strict mathematical or modeling sense, it seeks to identify irrigation levels that best support tree performance under real-world field conditions. This information is expected to guide horticultural practices for apple growers in similarly challenging agroecological zones.

2. Materials and Methods

2.1. Plant Materials and Experimental Site

The experiment was conducted during the 2020 cropping season at Faji Private Commercial Fruit Farm (*PLC*), located in Debre Birhan (Figure 1), in the central highlands of Ethiopia (2750 meters above sea level). The site receives approximately 900 mm of annual rainfall, concentrated in the main rainy season from June to September (Ethiopian National Meteorology Institute [*ENMI*], 2020). Daily mean temperatures range from 4–12°C during winter and 17–25°C in summer.

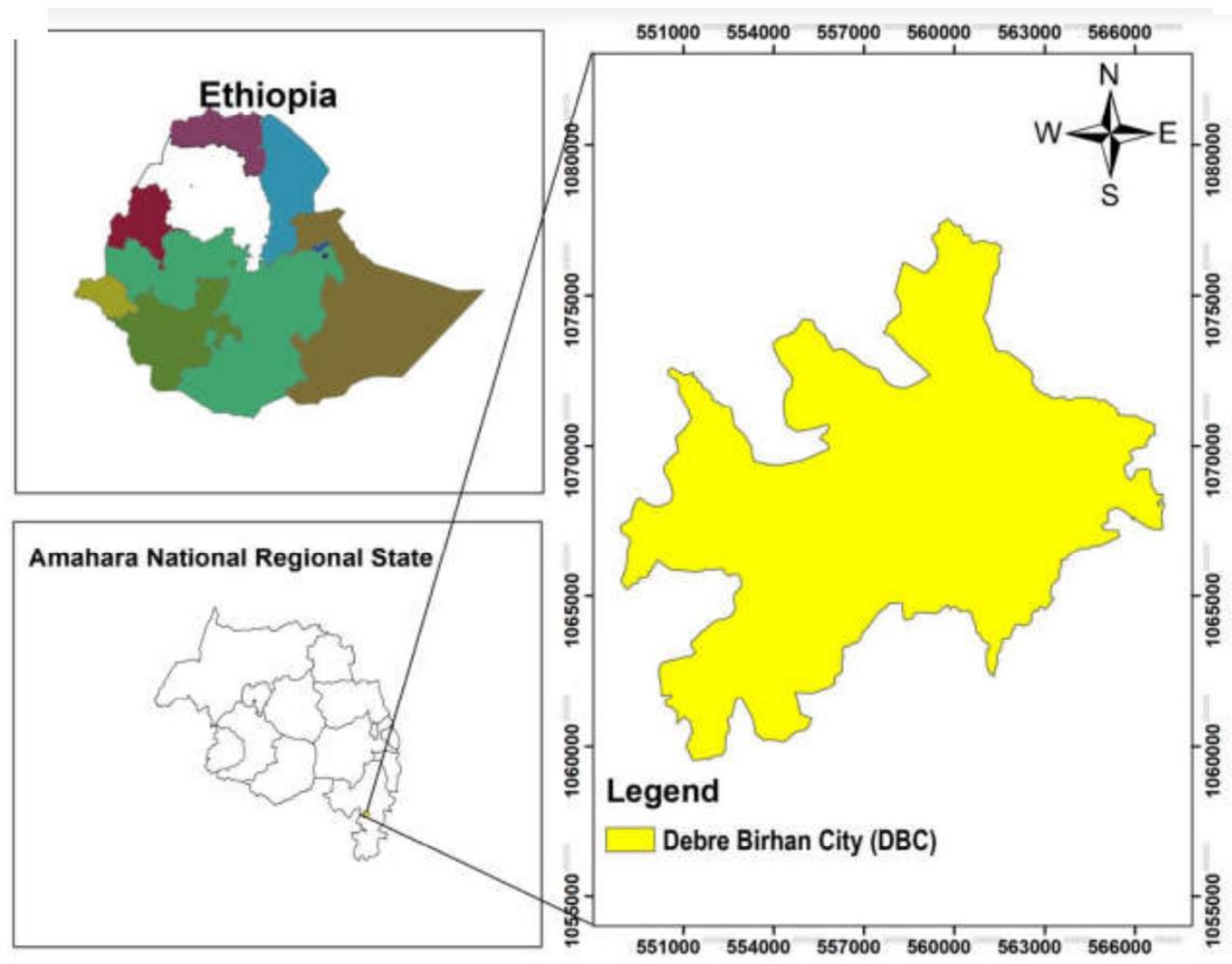

Figure 1. Study Location (Debre Birhan in Central Ethiopia)

The study used 7- years -old apple trees from two low-chill cultivars—Anna and Dorsett Golden—grafted onto MM-106 rootstock. These cultivars were selected for their suitability to highland conditions in Ethiopia, including effective bud break and flowering synchronization (Melke & Fetene, 2014; Labuschagne *et al.,* 2002).

2.2. Experimental Design and irrigation System

The orchard was established using a central leader training system, with trees spaced at 4 × 3 meters to optimize light interception and air circulation. The experiment employed a split-plot design within a randomized complete block design (*RCBD*), consisting of five replications. Each experimental plot contained eight trees. The main plots represented four irrigation levels, applied as percentages of crop evapotranspiration (*ETc*): 70%, 90%, 110%, and 130%. The subplots consisted of two apple cultivars—Anna and Dorsett Golden. The *ETc* values were calculated by adjusting the reference evapotranspiration (*ETo*) with crop coefficients (*Kc*) specific to apple trees. Reference evapotranspiration (*ETo*) was measured daily using the FAO Penman-Monteith method, which integrates meteorological data such as temperature, humidity, solar radiation, and wind speed from a nearby weather station. These *ETo* values were then multiplied by the crop coefficient (Kc), which varies throughout the growth stages of apple trees, to estimate *ETc* according to the formula:

$$ETc = ETo \times Kc$$

Where, *Kc* values varied depending on the crop's phenological stage. This method follows the guidelines provided by *FAO* (Fallahi et al., 2010).

Irrigation System

A drip irrigation system with two lines per row was installed, with each emitter delivering water at a flow rate of 2.5 L/h. Irrigation was applied based on crop water requirements calculated daily throughout the study period, with the frequency and amount adjusted according to the crop evapotranspiration (ETc).

The irrigation amount (I) in liters per day was estimated using the empirical formula:

$$I = ETc \times A \times 1000$$

Where, *ETc* (mm/day) is the crop evapotranspiration calculated by adjusting the reference evapotranspiration (*ETo*) from the Penman-Monteith method with crop coefficient (*Kc*), and *A* (m^2) is the ground surface area covered by the tree canopy or root zone.

Irrigation frequency was set daily to maintain optimal soil moisture and support tree growth, based on the high evapotranspiration demand under the tropical conditions of the study area. However, the volume applied per irrigation event was carefully controlled to avoid over-irrigation, considering the flow rate of emitters and the irrigation duration. The daily irrigation volume was derived from *ETc* values calculated using local *Kc* values specific to apple trees (Mebrie et al., 2023; Dragoni and Lakso, 2011; Glahn and Ruth, 2003), ensuring precise matching of water application to crop needs.

2.3. Data Collection

Weather data required for evapotranspiration (*ET*) calculations were obtained from on-site automated weather stations and cross-referenced with data from the National Meteorology Institute of Ethiopia to ensure accuracy. The Cornell Apple *ET* model (DeGaetano et al., 2015) was adapted to reflect site-specific conditions, including tree density and the timing of green tip emergence. Data were collected over a 240-day period, beginning from the bud swelling stage in October. Because, October marks the bud swelling stage**,** which is the start of the active growing season for apple trees in the study area. Beginning data collection at this time ensures that evapotranspiration calculations capture the full period of crop water use and development.

2.3.1. Phenological Data Collection

This period was categorized into three key phenological phases: (1) bud break to full bloom (0–90 days), (2) fruiting phase (90–180 days), and (3) vegetative rest (180–240 days). Meteorological data collected included daily average temperature, precipitation, and crop evapotranspiration (*ETc*). These variables were recorded consistently throughout the study period and are summarized in Figure 2 & Table 1).

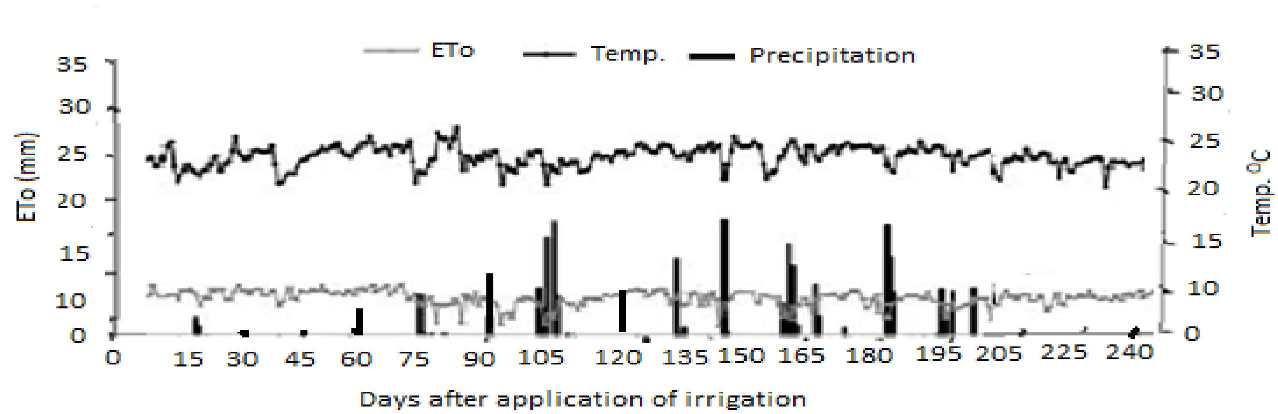

Figure 2. Climate data for experimental period (generated by Author).

Table 1. Monthly Climate Data for Debre Birhan Source: Ethiopian National Meteorology Institute (ENMI 2019) https://www.ethiomet.gov.et

Month	Avg Max Temp (°C)	Avg Min Temp (°C)	Avg Temp (°C)	Precipitation (mm)	Estimated ET_0 (mm/day)
January	20.0	8.0	14.0	12.18	3.5
February	20.0	10.0	15.0	29.70	3.6
March	21.0	11.0	16.0	106.43	4.0
April	21.0	11.0	16.0	169.72	4.2
May	21.0	12.0	16.5	162.40	4.1
June	21.0	12.0	16.5	175.44	3.9
July	18.0	11.0	14.5	530.00	3.2
August	17.0	11.0	14.0	565.99	3.0
September	18.0	10.0	14.0	329.09	3.3

October	19.0	9.0	14.0	60.28	3.7
November	19.0	8.0	13.5	18.18	3.8
December	19.0	8.0	13.5	8.09	3.6

Based on the crop development timeline for Debre Birhan, the climate data specifically average temperature, reference evapotranspiration (*ET_0*), and precipitation can be described across three key growth phases. Phase 1, from bud break to full bloom (vegetative stage), spans days 0 to 90, corresponding roughly to the months of January through March. Phase 2, the fruiting stage, extends from day 91 to 180, covering the period from April to June. Phase 3, the vegetative rest phase, occurs between days 181 and 240, approximately during July and August (Table 2). Each of these phases experiences distinct climatic conditions that influence crop water requirements and growth dynamics.

Table 2. Climatic Averages by Growth Phase

Phase	Avg Temp (°C)	Avg ET_0 (mm/day)	Total Precipitation (mm)
Vegetative (0–90 days) (Jan–Mar)	15.0	3.7	148.31
Fruiting (91–180 days) (Apr–Jun)	16.3	4.1	507.56
Vegetative Rest (181–240 days) (Jul–Aug)	14.25	3.1	1095.99

Notes: Temperature: Fairly stable throughout the period, slightly increasing in the fruiting phase; ET_0 Peaks in the fruiting phase due to higher radiation and drier atmosphere before the rains peak; Precipitation: Very low early in the season, then increases sharply, peaking in July–August (vegetative rest).

2.3.2. Physiological and Biochemical data

Gas exchange parameters were measured every 15 days using a portable infrared gas analyzer (IRGA; Li-Cor® 6400). The measured variables included net photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance *(g_s)*, internal CO_2 concentration (C_i), and instantaneous carboxylation efficiency (CE_i). These physiological indicators provided insights into the photosynthetic performance and water-use efficiency of the trees under varying irrigation levels and cultivar differences. Leaf chlorophyll content was estimated using a *SPAD*-502 chlorophyll meter (Minolta, Japan). Readings were taken from three fully expanded, sun-exposed leaves per tree, and the chlorophyll index (*LCI* %) was calculated to assess changes in leaf pigment concentration associated with plant health and productivity.

Relative water content *(RWC)* of leaves was determined following the method described in (Marsal et al., 2008):

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100$$

Where FM is the fresh mass, TM is the turgid mass, and DM is the dry mass.

For biochemical analyses, mature sun-exposed leaves were collected, immediately frozen in liquid nitrogen, and stored at −80°C until analysis. The assays included quantification of reducing sugars (*RS*) using the dinitrosalicylic acid (*DNS*) method, total soluble sugars (*TSS*) following the method described in Kumar et al., (2018) and total soluble proteins (*TSP*) determined by the Bradford method (1976), with bovine serum albumin (*BSA*) used as the standard. These biochemical markers provided critical information on the plant's metabolic adjustments under different physiological phases and irrigation regimes (Šircelj et al., 2005).

2.4. Statistical Analysis

All collected data were analyzed using Analysis of Variance (*ANOVA*). Mean comparisons were performed using Tukey's HSD test at a 5% significance level. Where significant irrigation effects were found, regression analyses were conducted to determine the trend response across irrigation levels. All analyses were conducted using SAS 9.4 statistical software.

3. Results

3.1. Apple Genotype Responses to Climate and Irrigation in Debre Birhan

The climatic data for Debre Birhan during the 240-day experimental period, encompassing the vegetative, fruiting, and vegetative rest phases, revealed distinct variations in temperature, reference evapotranspiration (*ETo*), and precipitation (Table 3). The vegetative phase (Days 0–90) experienced an average temperature of 15.0°C, with an *ETo* of 3.7 mm/day and total precipitation of 148.31 mm. In the fruiting phase (Days 91–180), the average temperature increased slightly to 16.3°C, *ETo* rose to 4.1 mm/day, and precipitation significantly increased to 507.56 mm. The vegetative rest phase (Days 181–240) saw a decrease in average temperature to 14.25°C, *ETo* decreased to 3.1 mm/day, and precipitation peaked at 1,095.99 mm. These variations in climatic factors are crucial as they directly influence the irrigation requirements and physiological responses of apple trees.

Table 3. Climatic data for the study location (Debre Birhan) during the 240-day experimental period

Phase	Days	Avg. Temperature (°C)	ETo (mm/day)	Total Precipitation (mm)
Vegetative	0–90	15.0	3.7	148.31
Fruiting	91–180	16.3	4.1	507.56
Vegetative Rest	181–240	14.25	3.1	1,095.99

Gas exchange measurements, including net photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), and instantaneous carboxylation efficiency (*CEi*), were recorded every 15 days throughout the study period (Table 4). These parameters exhibited significant fluctuations corresponding to the different phenological phases and irrigation levels. For instance, during the vegetative phase, the net photosynthetic rate was highest under the 110% *ETc* irrigation treatment, indicating optimal water availability for photosynthesis. However, as the study progressed into the fruiting and vegetative rest phases, variations in these physiological parameters were observed, reflecting the changing environmental conditions and irrigation treatments. These findings underscore the importance of tailored irrigation strategies to maintain optimal physiological function throughout the growing season.

Table 4. Gas Exchange Parameters at different irrigation levels

Irrigation Level (% *ETc*)	Net Photosynthesis (*A*) µmol CO_2 m^{-2} s^{-1}	Stomatal Conductance (*gs*) mol H_2O m^{-2} s^{-1}	Transpiration Rate (*E*) mmol H_2O m^{-2} s^{-1}	Internal CO_2 Concentration (Ci) ppm	Carboxylation Efficiency (*CEi*)
70%	10.4	0.19	3.2	248	0.042
90%	12.6	0.24	3.9	230	0.055
110%	14.8	0.30	4.5	217	0.068
130%	13.5	0.27	4.3	225	0.060

During the study period, gas exchange parameters demonstrated a strong response to irrigation levels. Net photosynthesis rates (A) increased progressively from 70% to 110% ETc, with the highest rate (14.8 µmol CO_2 m^{-2} s^{-1}) observed under 110% *ETc* (Table 4) This indicates that a moderate surplus of irrigation improves carbon assimilation, likely due to enhanced stomatal conductance and leaf hydration. However, a slight decline at 130% ETc suggests that over-irrigation or excessive application of water may begin to reduce photosynthetic efficiency, possibly due to transient hypoxic stress in the root zone.

Stomatal conductance (*gs*) followed a similar pattern, increasing significantly from 0.19 mol H_2O m^{-2} s^{-1} at 70% *ETc* to a peak of 0.30 at 110% *ETc* (Table 4). This trend reflects the plant's improved gas exchange capacity with adequate soil moisture, supporting optimal stomatal functioning. The slight reduction in *gs* at 130% *ETc* further supports the notion that excess water may disrupt physiological balance by affecting root respiration and nutrient uptake efficiency.

Transpiration rates *(E)* also increased with irrigation, reaching a maximum of 4.5 mmol H_2O m^{-2} s^{-1} at 110% *ETc* (Table 4). While transpiration is a critical cooling mechanism and nutrient transport pathway, excessive rates can also lead to water stress if not balanced by absorption. The observed trend suggests that 110% *ETc* supports the most efficient balance between water loss and gain under the given climate conditions. Correspondingly, internal CO_2 concentrations (*Ci*) declined with increasing irrigation, indicative of enhanced CO_2 fixation under better hydration conditions. Carboxylation efficiency (*CEi*), which combines aspects of both A and Ci, peaked at 110% *ETc*, reinforcing this level as optimal for physiological activity. Together, these findings highlight the pivotal role of irrigation in modulating physiological function in apple trees grown under tropical highland climates. While moderate deficit irrigation (70% and 90% *ETc*) constrained photosynthetic potential, over-irrigation (130% *ETc*) offered no additional benefit and may have posed subtle physiological drawbacks. This supports the conclusion that 110% *ETc* represents the most favorable irrigation regime for maintaining optimal gas exchange and water-use efficiency under the experimental conditions of Debre Birhan.

3.2. Chlorophyll Content and Relative Water Content (*RWC*)

Chlorophyll content, as measured by *SPAD* values, showed a clear positive response to increasing irrigation up to 110% *ETc* (Table 5). The lowest chlorophyll index was recorded under the 70% *ETc* treatment (mean *SPAD* value of 37.5), reflecting mild stress conditions likely induced by insufficient water supply. Under such conditions, leaf pigment degradation may occur more rapidly, reducing photosynthetic capacity. As irrigation levels increased, chlorophyll concentration improved significantly, peaking at 46.8 *SPAD* units under 110% *ETc*. This suggests that the additional moisture promoted sustained chlorophyll synthesis and delayed senescence.

A slight decrease in *SPAD* values at 130% *ETc* (mean 45.3) suggests a possible diminishing return or marginal stress effect, possibly due to water-logging or nutrient dilution in the soil. While the chlorophyll content at this level remained relatively high, the slight reduction compared to 110% *ETc* supports the interpretation that 110% is the optimal irrigation level for maximizing pigment content without over-saturating the root zone.

Table 5. Effect of Irrigation Levels on Chlorophyll Index and Relative Water Content.

Irrigation Level (% *ETc*)	**Chlorophyll Index (*SPAD* value)**	**Relative Water Content (*RWC*, %)**
70%	37.5	74.2
90%	42.1	80.6
110%	46.8	86.9
130%	45.3	84.1

Relative Water Content (*RWC*) (Table 5), showed a key indicator of plant water status and turgor maintenance, followed a similar trend. The lowest *RWC* value (74.2%) was observed at 70% ETc, indicating a moderate water deficit at the cellular level. As irrigation increased to 90% and 110% ETc, RWC improved significantly, reaching a peak of 86.9% at 110% *ETc*. This level likely maintained the best internal water balance, supporting physiological activity and cellular integrity. The slight decline to 84.1% at 130% *ETc* again reflects the subtle physiological cost of over-irrigation, possibly due to reduced oxygen availability in the root zone or changes in osmotic potential.

3.3. Biochemical Analysis

Biochemical assays measuring reducing sugars (*RS*), total soluble sugars (*TSS*), and total soluble proteins (TSP) were conducted on mature, sun-exposed leaves sampled at regular intervals (Table 6). The results indicated that irrigation levels influenced the accumulation of these biochemical compounds. For example, higher irrigation levels (110% and 130% *ETc*) led to increased concentrations of TSP, suggesting enhanced protein synthesis associated with improved water availability. Conversely, lower irrigation levels (70% and 90% *ETc*) resulted in reduced TSP concentrations, which may be indicative of water stress affecting protein metabolism.

Table 6. Effect of Irrigation Level (%)*ETc* on Reducing Sugars (mg/g FW), Total Soluble Sugars (mg/g FW) and Total Soluble Proteins (mg/g FW).

Irrigation Level (% ETc)	Reducing Sugars (mg/g FW)	Total Soluble Sugars (mg/g FW)	Total Soluble Proteins (mg/g FW)
70%	5.8	12.4	10.2
90%	6.7	14.6	11.8
110%	7.5	16.9	13.5
130%	7.1	16.1	12.9

The concentration of reducing sugars showed a significant increase with higher irrigation levels, peaking at 7.5 mg/g fresh weight (FW) under 110% *ETc*. (Table 6). Reducing sugars are closely tied to carbohydrate metabolism and are critical for maintaining osmotic balance and energy availability. Under limited irrigation (70% *ETc*), plants had the lowest reducing sugar content, reflecting a downregulation of photosynthate production or allocation under stress. The upward trend with increasing irrigation indicates enhanced metabolic activity and carbohydrate synthesis supported by improved leaf hydration and gas exchange efficiency. Total soluble sugars (*TSS*) followed a similar pattern. The lowest TSS concentration (12.4 mg/g FW) was observed at 70% *ETc*, likely due to restricted photosynthesis and limited translocation of assimilates.

Total soluble protein content also increased with rising irrigation levels, from 10.2 mg/g *FW* at 70% *ETc* to 13.5 mg/g *FW* at 110% *ETc.* Protein synthesis is highly sensitive to plant water status, as it is directly tied to nitrogen uptake and assimilation processes. The enhanced protein content at 110% *ETc* indicates a well-hydrated, metabolically active system with optimal nutrient uptake. The slight decline to 12.9 mg/g *FW* fewer than 130% *ETc* again suggests diminishing returns, where over-irrigation may marginally inhibit protein synthesis or dilute nitrogen concentration in the tissues. The present study indicates that biochemical parameters strongly support the trend observed in physiological responses: 110% *ETc* emerges as the optimal irrigation level**,** ensuring maximum accumulation of sugars and proteins without the metabolic penalties seen under deficit (70%) or excess (130%) irrigation. These results provide a robust basis for recommending irrigation strategies tailored to the climatic and soil conditions of tropical highland apple orchards.

3.4. Relationship among the studied Parameters across the tested Irrigation Levels

The response of apple trees to varying irrigation levels revealed a consistent trend across physiological, biochemical, and leaf water status parameters, with 110% of crop evapotranspiration (*ETc*) emerging as the optimal irrigation level (Table 7). Net photosynthesis rate (*A*), a direct indicator of carbon assimilation, increased steadily from 70% to 110% *ETc*, peaking at 14.8 µmol CO_2 m^{-2} s^{-1}, then slightly declining at 130%. This suggests that moderate surplus irrigation enhances gas exchange, likely due to improved leaf hydration and stomatal conductance (*gs*). The observed and predicted values closely matched, with high model accuracy ($R^2 \approx 0.99$), confirming the reliability of the trend. Similar patterns were noted in transpiration (*E*) and carboxylation efficiency (*CEi*), reinforcing the conclusion that 110% *ETc* supports optimal physiological functioning. Chlorophyll index (*SPAD*) and Relative Water Content (RWC) both increased significantly with higher irrigation, also peaking at 110% *ETc* with values of 46.8 *SPAD* units and 86.9% *RWC*, respectively. These values reflect improved photosynthetic pigment retention and better cellular hydration. A slight decline at 130% *ETc* suggests over-irrigation can subtly disrupt physiological processes, possibly due to reduced oxygen availability in the root zone. The predictive models again showed excellent fit, validating the observed trends.

Reducing sugars, total soluble sugars (*TSS*), and total soluble proteins (*TSP*) all followed a similar trajectory (Table 7). Their concentrations rose with increasing irrigation, peaking under 110% *ETc*—7.5 mg/g *FW* for reducing sugars, 16.9 mg/g *FW* for *TSS*, and 13.5 mg/g *FW* for *TSP*. These biochemical indicators reflect improved carbohydrate metabolism and protein synthesis under optimal hydration. The slight decrease at 130% *ETc* may be attributed to nutrient dilution or altered source–sink dynamics, common under excessive irrigation. Across all measured traits, 110% *ETc* consistently delivered the most favorable outcomes, suggesting it provides a balanced water supply that maximizes physiological performance, pigment stability, and biochemical activity without incurring the negative effects of water stress or over-irrigation. This optimal level aligns well with the unique climate and soil conditions of the tropical highland site in Debre Birhan, Ethiopia. Therefore, this study recommends 110% ETc as the ideal irrigation regime for apple production in similar environments, enhancing productivity while maintaining resource efficiency.

Table 7: Relationship among Physiological and Biochemical Parameters Across Irrigation Levels with R^2 Values

***ETc* (%)**	**Net Photosynthesis (A) µmol CO_2 m^{-2} s^{-1}**	***A* Predicted**	**Chlorophyll Index (*SPAD*) Units**	***SPAD* Predicted**	**Relative Water Content (*RWC*) (%)**	***RWC* Predicted**	**Reducing Sugars (*RS*) (mg/g *FW***	***RS* Predicted**	**Total Soluble Sugars (*TSS*) mg/g *FW***	***TSS* Predicted**	**Total Soluble Protein (*TSP*) mg/g *FW***	***TSP* Predicted**
70	10.4	10.6	37.5	37.9	74.2	74.8	5.8	5.9	12.4	12.5	10.2	10.4
90	12.6	12.5	42.1	42.4	80.6	81.0	6.7	6.8	14.6	14.7	11.8	11.9
110	14.8	14.7	46.8	46.5	86.9	86.7	7.5	7.5	16.9	16.8	13.5	13.4
130	13.5	13.4	45.3	45.2	84.1	84.3	7.1	7.0	16.1	16.0	12.9	12.8
R^2	–	**0.987**	–	**0.996**	–	**0.993**	–	**0.982**	–	**0.989**	–	**0.993**

4. Discussion

Phenological development of apple trees is intricately linked to water availability, which influences critical growth stages such as bud break, flowering, and fruit set (Mohawesh, and Al-Absi, 2009). In tropical highland regions like Debre Birhan, where climatic conditions differ from traditional apple-growing areas, irrigation plays a pivotal role in synchronizing these developmental phases. This study found that irrigation at 110% of crop evapotranspiration (*ETc*) resulted in optimal timing and progression of phenological events. This aligns with findings by González Nieto et al., (2024) and Quiroz et al., (2022), depicts that adequate irrigation enhances fruit growth rates and synchronizes developmental stages in apple trees under varying climatic conditions. Conversely, deficit irrigation (70–90% *ETc*) indicated in the present study led to delayed bud break and reduced flowering intensity. Šircelj, et al., (2007), and Bolat et al., (2014) indicates that insufficient water supply can disrupt the delicate balance required for proper phenological development. The present study also confirms that Over-irrigation (130% *ETc*) did not confer additional benefits and, in some cases, exacerbated physiological stress; and, can lead to quality deterioration, potentially making fruits more susceptible to disorders like bitter pit Kalcsits et al. (2019).

Physiological parameters such as net photosynthetic rate, transpiration rate, stomatal conductance, and internal CO_2 concentration are essential indicators of plant health and productivity (Wright et al., 2019). The present study demonstrated that irrigation at 110% ETc optimized these physiological processes, leading to enhanced photosynthetic efficiency and water-use efficiency. This is corroborated by findings from Robinson and Lopez (2012), who emphasized the importance of precise irrigation management in maximizing fruit productivity and quality in apples. Deficit irrigation resulted in reduced gas exchange rates, indicating water stress and impaired physiological function. Over-irrigation, while maintaining higher transpiration rates, did not translate into improved photosynthetic efficiency, highlighting the importance of balanced water supply. The present study further underscores the need for tailored irrigation strategies depending on the agroecological situations. While both Anna and Dorsett Golden cultivars exhibited improved physiological performance under 110% ETc irrigation, subtle differences in their responses suggest that genotype × irrigation interactions merit further investigation. Understanding these interactions can lead to more refined irrigation practices that cater to the specific needs of different apple cultivars, thereby enhancing overall orchard productivity.

Biochemical markers such as chlorophyll content (Zegbe & Behboudian, 2008), soluble sugars and proteins are indicative of the plant's metabolic status and its ability to adapt to environmental stresses (Tao et al., 2023; Cheng et al., 2004). This study found that irrigation at 110% *ETc* resulted in higher concentrations of these biochemical components, suggesting improved metabolic activity and stress resilience. Kalcsits et al. (2019) reported that regulated deficit irrigation strategies can enhance fruit nutritional status and quality by controlling tree vigor. Deficit irrigation led to lower concentrations of soluble sugars and proteins, indicating compromised metabolic function (Kalcsits et al. 2019). Over-irrigation did not significantly increase biochemical concentrations; reinforcing the notion that excessive water supply does not necessarily enhance plant metabolic activity. The implications of these findings are profound, as they suggest that maintaining optimal irrigation levels can improve not only the physiological performance but also the biochemical quality of apple fruits (Šircelj et al., 2007). This is particularly important in tropical highland regions, where climatic conditions pose unique challenges to apple cultivation. By adhering to irrigation practices that support both physiological and biochemical health, growers can enhance fruit quality and yield, contributing to the sustainability of apple production in these areas.

5. Conclusion

This study clearly demonstrates that irrigation levels significantly affect the phenological development, physiological performance, and biochemical responses of apple trees grown under tropical highland conditions in Debre Birhan, Ethiopia. Irrigation at 110% of crop evapotranspiration (*ETc*) consistently delivered the best results, with improved physiological efficiency, chlorophyll content, leaf water status, and higher soluble sugar and protein levels. Deficit irrigation (70–90% *ETc*) caused reductions in photosynthesis and metabolic activity, indicating stress, while irrigation beyond 110% *ETc* showed no additional benefit and seriously affects fruit productivity and quality. Incorporating phenology-specific crop coefficients (*Kc*) into *ETc* calculations is essential to align irrigation with actual crop water needs. Future research should include yield and fruit quality assessments to complement the physiological data. Additionally, integrating soil moisture monitoring and localized climate data can further refine irrigation efficiency.

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Declaration of interest

The author declares that no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. References

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