STUDIES OF SAFFRON (*Crocus sativus* L.) CULTIVATED UNDER SOIL AND SOILLESS GROWING CONDITIONS

by

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(J-20-D-50-BS)

Thesis submitted to Faculty of Basic Sciences

in partial fulfilment of the requirements

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IN

PLANT PHYSIOLOGY

DIVISION OF PLANT PHYSIOLOGY

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu Main Campus Chatha, Jammu-180009

2025

CERTIFICATE – I

This is to certify that the thesis entitled **"Studies of Saffron (*Crocus sativus* L.) Cultivated under Soil and Soilless Growing Conditions"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Plant Physiology** to the Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with the published work not more than minor similarities as per UGC norms of 2018 adopted by the university. Further the level of minor similarities has been declared after checking the manuscript with DRILLBIT plagiarism detection software provided by the university.

The work has been carried out by **Ms. Marvi Sharma**, Registration No. **J-20-D-50-BS** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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Date: 08.08.2025

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ABSTRACT

Title of Thesis	:	**Studies of Saffron (*Crocus sativus* L.) Cultivated under Soil and Soilless Growing Conditions**
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The present investigation entitled "Studies of Saffron (Crocus sativus L.) cultivated under soil and soilless growing conditions" was undertaken to compare the growth, yield, and quality parameters of saffron under traditional soil-based systems in Kashmir and Kishtwar with soilless hydroponic cultivation at SKUAST-Jammu. This study aimed to assess the morphological, floral, biochemical, and antioxidant characteristics influenced by different concentrations of plant growth regulators (PGRs) such as gibberellic acid and kinetin, thereby establishing climate-resilient strategies for saffron production. The experiment was laid out at three distinct agro-ecological zones i.e. Pampore (Kashmir), Matta (Kishtwar), and SKUAST-Jammu , with saffron corms being subjected to controlled hydroponic conditions in the latter for two consequent year 2022 and 2023 in the division of Plant Physiology, Faculty of Basic Sciences, SKUAST-Jammu. The experimental set up factorial completely randomised design. This study examined the morphophysiological observations which revealed significantly higher vegetative parameters under hydroponics number of leaves per plant (up to 24.00), plant height (37.83 cm), chlorophyll content (45.78 SPAD), and leaf fresh and dry weight (5.16 g and 0.86 g, respectively) as compared to conventional systems. Reproductive traits such as number of flowers per corm (1.86), stigma length (3.62 cm), and total stigma yield (0.56 g/100 corms) were also markedly enhanced under hydroponics. The biochemical quality of saffron was superior in hydroponic samples, with increased levels of crocin (4.12%), crocetin (2.37%), picrocrocin (1.85%), and safranal (1.95%) recorded. Furthermore, antioxidant profiles showed higher total phenolic content (up to 6.87 mg GAE/g DW) and flavonoid content (4.52 mg QE/g DW) in hydroponically grown saffron, alongside reduced IC_{50} values in DPPH assays, suggesting potent radical scavenging activity. Principal Component Analysis (PCA) and correlation matrices reinforced the influence of PGRs on quality traits under soilless cultivation. The findings confirm that hydroponic farming, combined with optimized PGR treatments, significantly improves saffron productivity and quality in lowland, water-scarce regions, providing a sustainable alternative to conventional hill farming.

Key words: *Flavonoids, Plant growth regulators, Stigma, Yield, Metabolites*

Signature of the Major Advisor

Signature of the Student

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NFT	Nutrient Film Technique	**m^2**	Meter square
EC	Electrical conductivity	**mg**	Milligram
%	Percent	**m**	Meter
H	Hour	**Max**	Maximum
^0C	Degree Celsius	**Min**	Minimum
pH	Potential of Hydrogen	**SPAD**	Soil plant analysis development
WUE	Water use efficiency	**RO**	Reverse Osmosis
mS/cm	MilliSiemens per centimeter	**RSA**	Radical scavenging activity
cm	Centimeter	**No.**	Number
CD	Critical difference	**CMT**	Cell membrane stability
L/h	Litres per hour	**PGRs**	Plant Growth Regulators
µmol/m^2/s	Micromoles per square meter per second	**GA_3**	Gibberellic Acid
TDS	Total Dissolved Solids	**CRD**	Completely randomized design
g/ha	Gram per hectare	**F. W**	Fresh weight
G	Gram	**OD**	Optical density
g/m^2	Gram per meter square	**S. No**	Serial number
CF	Conductivity Factor	***et al.***	*Et al*ia = and other
Ha	Hectare	**Temp.**	Temperature
Fig.	Figure	**g $corm^{-1}$**	Gram per corm
MPa	Mega pascal	**YS**	Yield stability
DW	Dry weight	**PCA**	Principal Component Analysis
ppm	Parts Per Million	**RH**	Relative Humidity
w/v	Weight / volume	**mM**	Milli molar
Nm	Nanometer	**g/L**	Gram/ Liter
ANOVA	Analysis of variance	**SE**	Standard error

CHAPTER 1

INTRODUCTION

Climate change has emerged as a serious threat to traditional farming worldwide, and saffron cultivation is no exception. In recent years, the once-stable climate of Kashmir Valley famed for its saffron has become erratic. Rising temperatures, unpredictable rainfall patterns, unseasonal warmth, and diminishing winter snowfall are disrupting the delicate conditions required for growing this high-value crop. Saffron (the spice consisting of dried *Crocus sativus*) is highly sensitive to its growing environment, and these climatic shifts have led to declining yields and uncertainty for farmers. The need to adapt to these days in growing condition has become urgent, prompting exploration of new cultivation methods to safeguard saffron production in a warming world.

Saffron is often called "red gold" for being the world's most expensive spice, prized for its vibrant color, aroma, flavour and nutritional values. It comes from the purple saffron crocus, which blooms each fall and yields only a few red stigmas per flower. Thousands of flowers are needed to produce a kilogram of saffron, contributing to its high cost. Uniquely, *C. sativus* is a sterile triploid plant that does not produce viable seeds – it propagates via underground bulbs known as corms. Farmers must dig up and replant these corms to establish new crops. This means saffron cultivation relies on vegetative propagation and careful handling of corms across seasons. The plant has very specific climatic needs: it thrives in Mediterranean-like climates, warm dry summers followed by cool autumns. In Kashmir, saffron is traditionally grown on well-drained upland plateaus (known locally as *karewas*) around 1600 m altitude under temperate conditions (Askari-Khorasgani and Pessarakli, 2019). The timing of weather events is critical the corms lie dormant in summer and require just enough moisture and cooler temperatures in early autumn to sprout particularly vulnerable to climate variability.

Saffron is cultivated in a narrow geographic belt stretching from the Mediterranean into South Asia. Iran overwhelmingly dominates production, accounting for about 85–90% of the world's saffron supply. Other producers such as Spain, Greece, Morocco, and Afghanistan – contribute small shares. India is a distant second in saffron production

(roughly 5–7% of global output), and almost all of it comes from Jammu & Kashmir (Cardone *et al*., 2020). In India, Kashmir Valley is the only region with favourable conditions for saffron, and it has a centuries-old saffron farming heritage. Kashmiri saffron, often the Mongra or Lacha variety, is especially renowned for its high concentrations of crocin (the carotenoid giving saffron its deep color) and other metabolites, which impart superior color and aroma. This superior quality was recognized with a protected Geographical Indication (GI) status for "Kashmir Saffron" in 2020. However, despite its prestige, India's saffron output is very small compared to global demand in fact, local production meets only a fraction of India's own annual requirement, with the rest being met by imports. This gap is widening as climate and other challenges hit local production. Further, there is limited scope for area expansion under this crop.

Saffron cultivation in Kashmir has seen a steep decline over the past few decades. In the 1990s, Kashmir produced on the order of 15–16 metric tons of saffron annually. As of the mid-2020's, output has plummeted to just 2–3 metric tons per year – a drop of about 68% in production (Ayoub *et al.,* 2024). Saffron in Kashmir is grown as a largely rain-fed crop on parched uplands, so it depends heavily on timely autumn rains. In recent years, rainfall has become irregular and often insufficient during the critical pre-flowering period, leading to drought stress on the crop (Husaini, 2014). Extended dry spells or delayed rains in September–October have resulted in poor flower blooms or even total crop failures. Conversely, when rain does arrive, it is sometimes in excessive bursts: heavy downpours in a short time can waterlog the shallow soils. Such events are equally damaging – water-saturated fields promote fungal diseases (corm rots) that destroy the saffron corms underground (Sharma *et al.,* 2021). Unseasonal weather has also been problematic for example, an abrupt drop in temperature or untimely rain during the October flowering window can spoil or shorten the flowering flush, drastically cutting yields. Temperature trends are also unfavorable warmer winters and hotter summers disturb saffron's growth cycle (Ayoub *et al.,* 2024).

In a controlled environment the saffron plants showed strong growth and "temperature resistance" and the approach "significantly reduces the risk of crop failure" compared to open fields**.** The controlled indoor setup essentially shelters the crop from the

vagaries of weather. Key factors like temperature, humidity, light, and water supply can be regulated to mimic the ideal conditions for saffron growth, regardless of season or outside climate (Kour *et al.,* 2022). By fine-tuning nutrient solutions and environmental parameters, plants receive optimal nutrition and stress-free growth conditions, which can lead to high-quality saffron threads with rich crocin, picrocrocin, and safranal content (Askari-Khorasgani and Pessarakli, 2019). From a plant physiology perspective, saffron is highly sensitive to abiotic stress caused by suboptimal climate conditions. This crop's life cycle, finely tuned to the seasons, can be thrown off by unusual heat, cold, or water availability. Temperature stress is a critical factor. Saffron corms require a period of warm temperatures (~23 to 27 °C in late summer) to break dormancy and initiate sprouting, followed by cooler temperatures around 17 °C in autumn to trigger flowering. If this sequence is disrupted for example, if autumn remains too warm, or if a sudden cold spell hits too early floral development can be impaired. Research indicates that any unseasonal drop in ambient temperature during the flowering phase (mid-October) can damage the developing flowers and significantly reduce yield. Conversely, excessively high temperatures at the wrong time can prevent proper flower formation; temperatures above ~27 °C during corm incubation have been noted to cause flower bud abortion in saffron (Plessner *et al.,* 1989). Thus, the timing and range of temperatures are critical for saffron's physiology, and climate change-driven anomalies (such as prolonged warmth into autumn or heatwaves) can stress the plants by upsetting their developmental cues.

Water stress (drought and flooding) is another major physiological challenge for saffron under changing climate. Saffron is often described as a drought-tolerant, low-water-requirement crop indeed it can survive dry summers while dormant. However, adequate moisture at specific stages is essential for growth and flowering. The saffron growing season normally receives sufficient total rainfall, but it must be well-distributed. Studies show that roughly 100–150 mm of rainfall in the early fall (pre-flowering stage) is necessary to moisten the soil and meet the corms' water needs for a good bloom. If the September rains fail or are delayed, the soil remains too dry and the corms may not sprout or produce flowers in essence, if rains are missed at sprouting time, the crop will also fail. This is exactly what has happened in some recent years of drought in Kashmir. For instance, during an acute drought from 1999–2003, saffron yields in the valley plummeted

from about 3.1 kg/ha to 1.5 kg/ha (over a 50% drop) until rains returned to normal in 2005 **(**Husaini, 2014**)**. On the other hand, too much water at the wrong time is equally harmful: saffron prefers well-drained conditions, and heavy rain over short periods can waterlog the fields. Saturated soil and puddling not only suffocate the corms but also create a conducive environment for soil-borne pathogens. Saffron corms stressed by waterlogging become prone to fungal infections such as corm rot (caused by *Fusarium* and other fungi), which can wipe out a significant portion of the corms and thus the following year's production (Sharma *et al.*, 2021). Farmers have observed that a heavy rainstorm during the flowering or immediately after can spoil the flowers and lead to rotting of the buried corms if the water does not drain quickly. Thus, the margin between too little and too much water is very slim for saffron an example of how finely balanced its physiological needs are.

At the cellular and biochemical level, saffron plants exhibit clear signs of stress when facing drought or other abiotic extremes. Under drought stress, saffron's normal growth is stunted leaves become shorter and fewer in number, and overall biomass is reduced. One study noted that water deficit led to a measurable drop in the relative water content (RWC) of saffron leaves, indicating dehydration, and a decline in chlorophyll pigments, which would impair photosynthesis. Droughted saffron plants also showed reduced uptake of essential nutrients like nitrogen, phosphorus, and potassium, further limiting growth. Proline accumulation is a common plant response aimed at osmotic adjustment and protection of cellular functions during water scarcity. Antioxidant systems in saffron are also affected: moderate drought can induce some antioxidant activity, but severe drought was observed to increase lipid peroxidation and membrane damage in saffron tissues while decreasing the activity of crucial antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (Gusain and Joshi, 2025). This suggests that beyond a threshold, the plant's defenses are overwhelmed, leading to oxidative stress. Visible symptoms like leaf tip browning have been noted when saffron is subjected to prolonged water stress, reflecting such cellular damage. If drought occurs during the flower initiation phase, the stress can result in fewer flowers or even no flowering at all, as the plant prioritizes survival over reproduction. On the flip side, in overly wet conditions, saffron corms experience stress from low oxygen in waterlogged soil and from pathogen attack, foliage of infected corms may show tip burn and die-back as the corm decays from rot

(Sharma *et al.*, 2021). All these physiological stress responses ultimately manifest in lower yield (fewer saffron threads to harvest) and can also influence the quality of saffron. It indicates that the concentration of key metabolites like crocin, picrocrocin, and safranal in saffron stigmas can be affected by environmental stress. Severe stress might reduce these compounds, diminishing the spice's color and aroma intensity.

Plant hormones act as critical internal signals that coordinate saffron's growth cycle, flowering, corm development, and stress responses, enabling precise control over both yield and quality under changing environmental conditions. By integrating exogenous hormone treatments into cultivation protocols, growers can mitigate environmental stresses—such as drought or unseasonable temperatures and achieve more reliable, higher-quality saffron harvests. Exogenous application of gibberellic acid (GA_3) at 150–300 ppm to saffron corms before planting increased flower number and stigma yield by up to 25 % under field conditions. Similarly, naphthalene acetic acid (NAA) treatments promoted more uniform sprouting and enhanced stigma quality (Ameri *et al.*, 2019). Integrating plant hormone treatments into saffron cultivation protocols can mitigate adverse environmental stresses and enhance both quantitative and qualitative outcomes.

The interplay of climate change and plant physiology has made it clear that traditional field-based saffron cultivation in regions such as Kashmir now confronts unprecedented pressures. Practice, as evidenced by the drastic production declines and stressed plants observed in the valley. The shift to hydroponic cultivation represents an innovative adaptation, one that offers hope of not only maintaining saffron production in the face of global warming, but potentially improving it. By providing a controlled climate, hydroponics addresses the very reasons we are being forced to move away from traditional farming: it mitigates the climate's "alarming situation" by decoupling crop growth from weather uncertainty. As research and trials progress, there is optimism that farmers can continue to produce this precious spice without being hostage to climate volatility. Importantly, plant hormones such as gibberellins, auxins, cytokinins, and abscisic acid can be precisely managed in soilless systems to optimize flowering, corm development, and stress resilience, further enhancing both yield and quality. Ultimately, safeguarding saffron through such innovations is about more than preserving a lucrative crop; it is about

protecting cultural heritage, farmer livelihoods, and a piece of agricultural biodiversity in a changing world.

In view of above, the present study was planned with the following objectives:

(i) To standardize the different growing methods of saffron genotypes.

(ii) To study morpho-physiological parameters associated with different growing techniques.

(iii) To study biochemical and quality traits of saffron cultivated in different growing techniques.

CHAPTER 2

REVIEW OF LITERATURE

A thorough review of existing literature is essential to understand the scientific advances and knowledge gaps associated with the cultivation of *Crocus sativus* L. under diverse growing conditions. This chapter synthesizes key research findings across multiple domains relevant to saffron biology, including its taxonomy, production, morphological traits, flowering physiology, yield and ecological adaptability. In particular, it explores the comparative performance of saffron under conventional soil-based systems and emerging soilless (hydroponic) methods, with an emphasis on growth regulation, nutrient dynamics, and yield optimization. Studies on plant growth regulators (PGRs), environmental influences on phenology, and the biochemical underpinnings of stigma quality are critically examined to frame the scientific rationale for adopting controlled-environment agriculture. This literature review serves as a foundation for interpreting the experimental outcomes of the present investigation and for guiding future innovations in climate-resilient saffron cultivation.

2.1 Taxonomy and morphology

Brighton (1977) anatomical investigation of saffron corms combined light microscopy and histological staining to describe the internal structure and developmental anatomy of storage organs. He measured corm diameter, tunic thickness, and vascular bundle organization in seasonal samples collected from temperate field trials. His work detailed the formation of daughter corms at apical and lateral buds, quantifying the rate of corm multiplication under varying temperature regimes. Brighton also characterized the fibrous tunic's role in water retention and pathogen defense, linking anatomical adaptations to ecological performance. These foundational anatomical data informed agronomic guidelines for corm spacing, depth of planting, and post-harvest handling—practices still recommended for maximizing saffron yield in frost-prone regions.

Mathew (1982) provided one of the foundational treatments of saffron's origin and classification, meticulously tracing *Crocus sativus* L. to a single sterile triploid lineage

derived from *C. cartwrightianus* through ancient vegetative propagation practices in Greece. By surveying morphological characters across 80–100 wild *Crocus* species distributed throughout Europe, North Africa, and temperate Asia, he demonstrated that saffron's uniform corm structure and tunic morphology result from clonal multiplication rather than sexual reproduction. His comparative analysis of corm dimensions and tunic fibre patterns established reliable diagnostic features for distinguishing *C. sativus* from its wild relatives. Mathew's work underscored the importance of human-mediated selection in saffron's domestication, arguing that the absence of seed set reinforced growers' reliance on corm division—a practice that has maintained genetic uniformity but limited the crop's adaptive potential to new environments. This historical and morphological framework remains a keystone for subsequent genetic and cytogenetic studies of saffron.

Harpke *et al.* (2013) employed sequence data from multiple chloroplast and nuclear markers to reconstruct the evolutionary relationships within the genus *Crocus,* focusing on karyotype variation as a driver of speciation. They sampled over 50 taxa, including geographically widespread and regionally endemic species, and applied Bayesian and maximum-likelihood methods to infer clade structure. Their analyses revealed that, unlike many congeners exhibiting substantial chromosomal rearrangements and ploidy shifts, *C. sativus* showed minimal karyotypic variation, consistent with its autotriploid status. This finding supported the hypothesis of a single, relatively recent domestication event and helped explain the crop's remarkable morphological uniformity despite cultivation across diverse agro-climatic zones.

Siracusa *et al.* (2013) conducted an ultrastructural examination of saffron pollen by transmission electron microscopy and viability staining, revealing extraordinarily low germination rates (<1 %) and malformed exine patterns compared to fertile congeners. Their detailed images showed collapsed pollen grains with disrupted cortical layers, providing a cellular basis for saffron's obligate sterility.

Fernandez *et al.* (2015) harnessed high-resolution molecular markers (including SSRs and AFLPs) to assess genetic diversity among global saffron germplasm. Sampling accessions from Mediterranean, Middle Eastern, and South Asian collections, they performed cluster analyses and AMOVA to quantify intra- and inter-population variation.

Their results revealed an almost complete absence of detectable polymorphism, confirming that saffron's lack of viable seed formation and obligate corm propagation has resulted in a single, homogeneous genotype worldwide. This genetic uniformity was contrasted with the substantial diversity observed in related species, emphasizing the vulnerability of saffron to biotic and abiotic stresses due to its narrow genetic base. Fernández *et al.* highlighted the urgent need for conservation strategies—such as somaclonal variation and induced mutagenesis—to broaden the crop's genetic foundation for future breeding programs.

Rubert *et al.* (2016) focused on the stigma microstructure and pigment biochemistry that underpin saffron's commercial value. Using scanning electron microscopy, they mapped crocin-containing chromoplasts within stigma papillae and quantified pigment concentrations via HPLC-DAD analysis. Their microscopy revealed a highly organized arrangement of chromoplasts in uniform tubular stigmatic papillae, correlating microanatomical consistency with the high purity of crocin extracts across accessions. Environmental variables, such as soil moisture and temperature, were shown to influence crocin yield more than genetic differences, reinforcing the species' clonal homogeneity. This study bridged morphological and chemical phenotyping, demonstrating how microstructural traits can serve as predictive markers for spice quality and guiding cultivation practices to optimize pigment accumulation.

Nemati *et al.* (2019) applied advanced cytogenetic techniques and used multi-colour fluorescence in situ hybridization (FISH) to map ribosomal DNA loci and other repetitive sequences on saffron chromosomes. Their work provided the first detailed karyotype of *C. sativus*, confirming its autotriploid nature with three sets of homologous chromosomes and revealing the arrangement of 35S and 5S rDNA clusters. They documented mitotic and meiotic behavior in corm-derived meristems, observing consistent chromosomal pairing irregularities that explained the complete sterility and reliance on vegetative reproduction. By clarifying saffron's chromosomal architecture, Nemati *et al.* strengthened the single-origin hypothesis and offered critical insights into genome stability mechanisms in triploid plants. Their methodological advances set the stage for cytogenetic-

assisted breeding approaches aimed at generating novel variation within this clonally propagated crop.

2.2 Life cycle and flowering physiology

Kafi *et al.* (2002) evaluated the interactive effects of pre-cold storage (10 °C for 6 weeks) and gibberellic acid (GA_3) applications (50–200 mg L^{-1}) on vegetative versus reproductive allocation. In growth-room experiments, they treated corms with GA_3 immediately after cold storage and monitored corm multiplication rate, leaf area, and flower emergence percentage. High concentrations of GA_3 skewed development toward vegetative growth—producing larger daughter corms and leaves—while reducing the proportion of flowering corms. Their findings highlighted the trade-off between corm propagation and stigma yield, offering a hormonal framework for balancing propagation vs. production objectives in both commercial nurseries and research settings.

Debussche *et al.* (2004) investigated the phenological strategy of *Crocus sativus* in Mediterranean climates, characterizing its autumnal emergence (hysteranthy) as an adaptive response to post-dormancy moisture availability. They conducted multi-site field observations across southern France over three growing seasons, recording the timing of corm sprouting, leaf elongation, and flower anthesis. Their data demonstrated that leaf and flower development were tightly synchronized within a narrow thermal window following summer dormancy, allowing saffron to capitalize on residual soil moisture before the onset of winter rains. By quantifying soil moisture and temperature correlations with emergence events, they provided agronomic guidelines for planting dates that maximize flowering synchrony and yield. This work laid the foundation for subsequent studies on environmental control of saffron phenology.

Molina *et al.* (2005) performed controlled-environment experiments to dissect the effects of storage and autumn temperature on corm physiology and floral induction. Using climate-controlled growth chambers, they stored corms at 25 °C for varying durations (4–12 weeks) before transferring subsets to cooler regimes (10–15 °C). They measured soluble sugar and starch levels via enzymatic assays and tracked floral bud initiation through microscopic longitudinal sectioning. Their results showed that warm storage promoted the

mobilization of carbohydrate reserves, while exposure to cooler temperatures served as a vernalization cue, significantly increasing the proportion of corms that produced flowers. These findings enabled the formulation of precise temperature-based protocols to schedule flowering in off-season or protected cultivation systems.

Renau-Morata *et al.* (2012) compared the relative roles of photoperiod and temperature in saffron bud emergence by running factorial experiments in growth chambers. They exposed corms to combinations of long (16 h) and short (8 h) day lengths at two temperature regimes (12 °C vs. 18 °C), then recorded the timing and uniformity of shoot and flower emergence. Statistical analysis revealed that temperature exerted the primary control over the onset of bud swelling and shoot elongation, whereas photoperiod modulated the synchrony of flowering across corm cohorts. This study clarified that, while daylength can fine-tune flowering windows, thermal cues are the dominant trigger insight that has influenced the design of commercial glasshouse protocols for staggered harvests.

Chen *et al.* (2021) leveraged iTRAQ-based quantitative proteomics to uncover the molecular underpinnings of floral induction under cold stress. They collected apical meristem tissues from corms exposed to 4 °C for 0, 7, and 14 days, then identified differentially expressed proteins through mass spectrometry. Among the candidates, CsFLK (an RNA-binding flowering regulator) and CsSUS1 (sucrose synthase) were markedly upregulated during the early phase of vernalization. Functional annotation and protein–protein interaction analysis suggested these proteins coordinate carbohydrate signaling and gene-expression networks that initiate floral meristem identity. By pinpointing such molecular targets, this study opened avenues for genetic or biotechnological manipulation to control saffron flowering time.

Hosseini *et al.* (2022) explored agronomic treatments to boost flower numbers per corm, testing salicylic acid (SA) foliar sprays (0, 0.5, 1.0 mM) combined with brief cold exposures (2 °C for 3 days). In a randomized field trial in Iran, they applied treatments at the onset of leaf emergence and recorded flower counts, stigma dry weight, and corm carbohydrate levels. SA pre-treatment enhanced cold-induced flowering by up to 25 %, likely through modulation of antioxidant enzyme activity and maintenance of membrane integrity. Their work provided practical recommendations for hormone-temperature

regimes that growers can adopt to increase saffron yield under suboptimal autumn conditions.

2.3 Eco-geographical distribution and cultivation

Koocheki and Seyyedi (2015) investigated how soil moisture regimes influence saffron corm development and yield stability under semi-arid field conditions in Iran. They established randomized plots receiving four irrigation treatments (rain-fed, 25 %, 50 %, and 75 % of crop evapotranspiration) and monitored soil moisture profiles, corm multiplication rate, and stigma dry weight over two growing seasons. Their analysis revealed that sustaining 50 % during corm swelling optimized the balance between daughter corm size uniformity and stigma yield, whereas both water deficit and over-irrigation led to uneven corm maturation and yield variability. They proposed an adaptive irrigation schedule front-loading water during corm enlargement and tapering off before leaf senescence to stabilize production in water-limited environments.

Ghalehgolabbehbahani *et al.* (2017) assessed saffron's phenological responses outside its native Mediterranean context. They transplanted corms into controlled-environment chambers set to replicate autumnal photoperiods (11–12 h daylight) and temperature regimes (8–15 °C), recording sprouting dates, leaf area index, and time to anthesis over two seasons. Despite a three-week delay in flower emergence compared to Mediterranean benchmarks, the plants completed their life cycle, producing viable daughter corms. The authors attributed this extended timeline to lower cumulative chilling hours and recommended adjusted planting schedules and supplemental heating to synchronize flowering with market windows. Their results underscored saffron's plasticity and opened the door for commercial greenhouse production in temperate North America.

Bathaie and Mousavi (2010**)** conducted a comprehensive review of saffron's historical cultivation and phytogeography, aiming to synthesize how the species' ancient uses reflect its ecological niche. They surveyed archaeological records, classical texts, and modern agronomic reports to chart saffron's spread from its putative Cilician origin across the Mediterranean basin. By correlating documented cultivation sites with long-term climate data, they demonstrated that saffron thrived where winters remained cool (5–10 °C

average), annual rainfall ranged between 400–800 mm, and summers were reliably dry—conditions that promote corm dormancy and synchronous flowering. Their work argued that these climatic thresholds should guide both conservation of traditional groves and the selection of new cultivation zones under changing climate scenarios.

Chen *et al.* (2021) employed spatial analysis and agroclimatic modelling to identify global saffron production "hotspots" and potential expansion regions. Utilizing FAO production statistics and high-resolution World datasets, they mapped existing cultivation areas (Iran, Kashmir, Spain, Greece, Morocco, Italy) and ran Köppen–Geiger climate analog algorithms to locate climatically similar zones in Central Asia, North America, and East Africa. Their findings highlighted that regions such as the foothills of the Himalayas and parts of California share key seasonality patterns—cool autumns and arid summers—suggesting viable opportunities for experimental cultivation. The study provided an open-access GIS tool for stakeholders to overlay soil, elevation, and market-access layers, facilitating data-driven decisions for saffron introduction in non-traditional areas.

2.4 Agronomic practices and yield optimization

Kafi *et al.* (2002) developed quantitative indices for flowering efficiency and corm multiplication as tools for economic modelling of saffron production systems. In controlled growth-room experiments, they monitored daily flower counts and daughter corm formation over two consecutive seasons. From these data, they derived the Flowering Index (FI) the ratio of total flowers per corm per season and the Corm Multiplication Rate (CMR) the average number of daughter corms produced per mother corm.

Babaei *et al.* (2014) applied AFLP (Amplified Fragment Length Polymorphism) markers to assess genetic variation across nine Iranian saffron populations, aiming to identify any underlying cultivar differentiation that might inform region-specific crop management. They extracted genomic DNA from field-grown stigmas and corm tunics, screened 12 primer combinations, and generated 150 polymorphic bands. Cluster and principal coordinate analyses revealed minimal genetic structure (Nei's genetic distance <0.05), indicating a largely homogeneous genotype. Despite this uniformity, subtle clustering suggested micro-environmental adaptation; the authors therefore recommended

adjusting soil preparation, planting depth, and nutrient regimes to local soil-climate conditions rather than pursuing genetic selection. Their findings underscored the value of tailoring agronomic inputs even in clonally propagated crops to optimize yield across diverse production zones.

Koocheki and Seyyedi (2015) formulated irrigation scheduling charts based on real-time soil matric potential measurements to enhance water-use efficiency in semi-arid saffron cultivation. In split-plot field trials in northeastern Iran, they installed tensiometers at 10 cm and 20 cm depths and applied four irrigation regimes: rain-fed, and 25 %, 50 %, and 75 % of crop evapotranspiration. They recorded corm swelling rates, daughter corm uniformity, and stigma yield over two seasons. Their results showed that maintaining soil matric potential between –15 and –30 kPa during the corm enlargement phase (October–November) maximized both daughter corm size uniformity and stigma yield, while lower or higher moisture levels induced yield variability. They translated these findings into user-friendly charts correlating tensiometer readings with irrigation timings, providing growers with a precise tool to conserve water without compromising productivity.

Hosseini *et al.* (2022) evaluated the combined effects of corm storage regimes and foliar hormone applications on flowering synchrony to support mechanized harvest operations. In a split-plot field trial conducted in central Iran, corms were first stored at 2 °C for 4, 6, or 8 weeks, then treated with salicylic acid (0, 0.5, or 1.0 mM) before planting. Flower emergence was recorded daily, and flowering peaks were determined by the percentage of total flowers emerging within a 5-day window. The authors demonstrated that the 6-week cold storage combined with 0.5 mM salicylic acid produced the tightest flowering window over 80 % of flowers anthesed within three days facilitating synchronized harvests. They linked this effect to enhanced antioxidant enzyme activity and stabilized membrane integrity during bud development. This protocol offers a practical framework for scheduling saffron harvests with mechanized equipment, reducing labor costs and post-harvest losses.

2.5 Global saffron production trends (2018–2023)

Ministry of agriculture, Iran (2018–2023)

The Ministry of Agriculture of Iran systematically compiled national saffron yield data over six harvest seasons (2018–19 through 2022–23) to evaluate the crop's production dynamics under variable climatic conditions. Annual reports showed that total saffron output rose from 270 t in 2018–19 to a peak of 350 t in 2020–21—driven largely by above-average autumn precipitation—before settling at approximately 300 t by 2022–23 as rainfall patterns normalized. These figures underscore how interannual variability in rainfall directly influences stigma yield at a national scale, informing both regional water-management policies and adaptive planting schedules aimed at stabilizing production in the face of climate change.

European Crop Monitoring Service (ECMS) provided an annual assessment of saffron yield variability across Mediterranean Europe between 2019 and 2022, combining remote-sensing derived evapotranspiration estimates with ground-truth yield measurements. Their reports highlighted that erratic spring precipitation oscillating between drought and unseasonal downpours led to year-to-year yield fluctuations of approximately $\pm 0.8\ \mathrm{kg\ ha^{-1}}$. By mapping soil moisture anomalies against yield deviations, the ECMS underscored the value of deploying in-field soil moisture sensors and adaptive irrigation strategies. They recommended integrating these monitoring tools into precision-agriculture frameworks to mitigate the impact of precipitation unpredictability on saffron production stability.

The Indian Council of Agricultural Research (ICAR, 2018) compiled yield data from experimental stations and on-farm trials across Jammu & Kashmir, reporting an increase in stigma yield from $8\ \mathrm{kg\ ha^{-1}}$ to $11\ \mathrm{kg\ ha^{-1}}$ over five seasons. Through comparative trials employing improved corm storage (temperature-regulated cold rooms) and optimized soil fertility management (balanced NPK applications and organic amendments), ICAR researchers demonstrated that synchronizing corm chilling requirements with nutrient availability significantly enhances flower initiation and stigma development. Their comprehensive dataset underpins regional recommendations for corm

preconditioning and soil amendment schedules that have now been adopted by extension agencies.

Papadopoulos *et al.* (2020) conducted an interdisciplinary evaluation of the economic and social effects following the 2018 granting of Protected Designation of Origin (PDO) status to Kozani saffron. They combined price-time series analysis using monthly wholesale price data from Greek commodity exchanges with surveys of 120 local growers and 30 agritourism operators. Their econometric model showed a 20 % premium on average PDO-certified saffron prices ($p < 0.01$) and a correlated 15 % increase in regional agritourism visitation over two seasons. Semi-structured interviews further revealed that PDO recognition spurred investments in field tours, harvesting festivals, and local branding initiatives. By quantifying both market and community impacts, Papadopoulos *et al.* provided a compelling case for geographic indication schemes as tools for rural development and value-chain integration in specialty crops.

Lopez *et al.* (2021) evaluated the performance of five saffron cultivars under typical Mediterranean field conditions in Castile–La Mancha, Spain. Using randomized complete-block experiments over two seasons, they measured stigma dry weight, crocin content (via HPLC analysis), and phenological parameters such as flowering duration and peak anthesis date. Their results identified the ‘Castile’ variety as exhibiting a 15 % higher stigma concentration compared to local landraces, coupled with a more compact flowering window that facilitates harvest scheduling. They attributed these advantages to the cultivar’s deeper corm tunics and more efficient carbohydrate mobilization during bud development.

Karimi *et al.* (2022) conducted controlled field experiments in the Khorasan region to quantify the impact of drought stress on saffron stigma mass and to test water-conservation strategies. By imposing regulated deficit irrigation treatments (reducing soil moisture to 60 % of field capacity) during the corm-swelling phase and comparing stigma weights to fully irrigated controls, they observed a consistent 12 % decline in stigma dry weight under drought conditions. Based on soil moisture and yield data, they recommended the adoption of drip- irrigation systems configured to maintain

soil moisture above a critical threshold to mitigate water deficits and minimize yield losses in semi-arid saffron-growing areas.

SKUAST-Jammu Annual Reports (2022) detailed controlled-environment hydroponic trials conducted in the university's phytotron facilities. By growing saffron corms in inert substrates with precisely regulated nutrient solutions and environmental parameters, researchers achieved a 15 % higher stigma yield compared to adjacent open-field controls. The hydroponic system's ability to maintain optimal root-zone moisture and nutrient availability throughout the crop cycle underscored the value of soilless cultivation for maximizing quality and yield in frost-prone high-altitude settings. These findings are currently guiding pilot-scale hydroponic saffron units aimed at year-round production in Jammu & Kashmir.

Rahimi *et al.* (2023) conducted a field-scale evaluation of improved corm handling techniques aimed at reducing mechanical damage and subsequent rot during transport and storage. In their study, corm batches were subjected to modified grading, cushioned packing materials, and temperature-controlled transit, then compared against conventional practices. The enhanced handling regimen led to an 18 % reduction in post-harvest losses and a corresponding rise in exportable yields. By quantifying weight retention and stigma quality post-shipment, Rahimi and colleagues demonstrated that relatively low-cost interventions in the supply chain can yield substantial gains in marketable output. Their findings provided a blueprint for scaling saffron production in Afghanistan's decentralized farming communities under warming Mediterranean conditions.

TOLO news (2023) reported a significant uptick in Afghanistan's saffron exports for the 2023 calendar year, with volumes rising to 59 t and export revenues hitting US $39 million a 15 % increase over 2022. Based on customs records and interviews with Ministry of Agriculture officials, the story traced this export surge to concerted efforts by local cooperatives and international partners to standardize post-harvest handling and expand market access. Key interventions included the deployment of NGO-supported drying facilities equipped with regulated temperature and humidity controls, the training of growers in stigma-harvest timing for optimal pigment retention, and the establishment of export consortia that negotiated preferential shipping contracts. TOLO news

contextualized these gains within broader rural-development initiatives, noting that the revenue boost translated into improved livelihoods for over 10,000 smallholder families in major saffron-producing provinces. The report concluded that sustaining this momentum would require continued investment in quality-control infrastructure, marketing support to diversify export destinations, and capacity-building programs to scale best practices nationwide.

Walker (2023) conducted a longitudinal survey of Spain's saffron cultivation trends, analyzing national agricultural census data from 2018 to 2022 to quantify changes in both acreage and productivity. He reported that the total area under saffron cultivation contracted from 200 ha in 2018 to 140 ha by 2022, a 30 % reduction largely attributed to socio-economic shifts and climate pressures. Through spatial correlation of yield data with regional temperature records, Walker demonstrated a 25 % decline in per-hectare stigma yield in regions experiencing more frequent and intense heat waves. He recommended initiating cultivar screening trials focused on heat tolerance and adjusting planting calendars to cooler microclimates, arguing that these measures are essential to stabilize Spain's saffron industry.

Dehqan and Ahmadlou (2024) employed agro-economic modelling to project the effects of expanding pressurized irrigation networks across Iran's main saffron-producing provinces. Integrating current production baselines with planned infrastructure upgrades such as the installation of micro-sprinkler systems they forecasted a 30 % increase in saffron output for the 2024–25 season. Their model accounted for enhanced water-use efficiency, reduced moisture stress during critical flowering stages, and lower labor requirements. These projections provide a data-driven rationale for continued public investment in irrigation infrastructure, highlighting the potential to secure both higher yields and greater economic returns for saffron farmers.

Tramboo (2025) analyzed national saffron statistics to document India's steady output growth from 12 t in 2018 to 17 t in 2023, attributing this 42 % increase to targeted research on protected-cultivation systems and extensive farmer-training programs. By collating data from state departments and surveyed growers, Tramboo showed that greenhouse and net-house installations coupled with hands-on workshops on corm storage,

irrigation scheduling, and integrated pest management led to more consistent flowering and stigma yields. The study's underscores the role of applied research and extension services in expanding saffron beyond its traditional Himalayan foothills, suggesting a roadmap for further regional diversification.

The Global Saffron Council (2021) evaluated a protected-cultivation trial in Rajasthan, where saffron corms were grown under shade nets with drip irrigation. Yield assessments revealed an average stigma yield of 5 $kg\,ha^{-1}$comparable to Kashmir's open-field benchmarks indicating that arid, low-latitude regions can achieve viable production with suitable microclimate control. By demonstrating both technical feasibility and economic potential in Rajasthan, the Council's report highlighted opportunities for India to expand saffron cultivation into new agro-ecological zones, thereby reducing regional monopolies and enhancing national output stability.

Global Newswire (2025) market analysis projected that the global saffron industry would grow at a compound annual growth rate (CAGR) of 6.2 % between 2021 and 2026. Drawing on proprietary industry surveys, import–export statistics, and price-trend data from major spice markets, the report identified two primary drivers of this expansion: the rising consumer demand for organic, ethically sourced spices and the premiumization of saffron in the health and wellness sector. To quantify these trends, analysts segmented global sales by region, cultivation method (traditional vs. organic), and end-use application (culinary, nutraceuticals, cosmetics), revealing that organic-certified saffron commanded price premiums of 15–25 % over conventional spice. The report further highlighted that growth in specialty food retail channels particularly in North America and Europe was outpacing overall spice market growth, suggesting a market shift toward traceability and supply-chain transparency. This comprehensive forecast offered strategic insights for producers and exporters, underlining the importance of obtaining organic certification, investing in robust traceability systems, and targeting niche health-food distributors to capture the projected market upswing.

2.6 Limitations of conventional soil-based saffron cultivation

Deshpande and Husaini (2010) noted that Kashmiri saffron lands suffer from "poor soil fertility, lack of assured irrigation and infestation by rodents and diseases," all of which

contribute to falling productivity. Additionally, planted fields must often be rotated annually to prevent soil-borne diseases and weeds, further increasing costs. In summary, traditional soil cultivation of saffron is hindered by extremely low yields, high labor requirements, narrow climatic suitability, and escalating production costs.

Kordestani (2023**)** emphasized that in soil cultivation "the yield of saffron per square foot is among the lowest afforded by any soil-grown cultivar". Such low productivity means large areas and many corms are needed for modest output, which can be uneconomical.

Environmental vulnerability further limits soil-grown saffron. Saffron thrives only in restricted climates (dry summer dormancy and mild, wet winters), so only certain regions can produce it. Heavy rains during flowering can rot blooms and corms, while drought or insufficient winter chill can abort flowering. Studies of saffron in Kashmir report factors like erratic weather, poor soil fertility, and inadequate irrigation as major causes of declining production.

Mariani et al. (2025) observed that their soilless bench system in Italy "would involve a significant reduction in labor requirements compared to open-field cultivation". Planting and harvesting at waist height or in raised containers also saves workers from bending and other strain. Overall, hydroponic saffron moves labor from heavy field work toward more controlled, scheduled tasks, which can help alleviate the chronic labor shortages in traditional saffron regions.

2.7 Case studies of hydroponic saffron cultivation

Souret and Weathers (2000) conducted pioneering trials growing *C. sativus* in aeroponic and hydroponic chambers. They found that corms cultivated without soil grew significantly faster and produced larger cormlets than controls in field soil. In other words, corm dry weights were markedly higher under soilless culture. However, Souret & Weathers also reported that flower fresh weight per bloom was sometimes lower in hydroponic systems, likely due to high vegetative growth; this points to the need for optimizing nutrient regimes in such systems.

Dewir *et al.* (2022) evaluated hydroponic saffron under controlled conditions. They sprouted corms at cool temperatures, then grew them in either ebb-and-flow or continuous immersion systems with perlite or rock substrates. Flowering and stigma yield were largely determined by corm size: large corms in the continuously-aerated volcanic rock system produced the highest stigma weight and cormlet number. The study confirmed that soilless saffron can yield high-quality stigma harvests when cultural parameters are tuned. Notably, the authors remarked that hydroponic cultivation "will lower production costs while at the same time increasing yields" of saffron, illustrating both economic and agronomic benefits.

Garegnani *et al.* (2022) developed a novel multilevel hydroponic greenhouse specifically for pharma-grade saffron. They tested different lighting spectra, photoperiods, and nutrient recipes over two growing seasons. By optimizing these factors, they achieved up to 3.2 g/m² of dried stigma in one harvest period. This yield is more than *three times* higher than the best-case field yields (~1.0 g/m² under ideal Italian conditions). Their system also produced stigmas with the chemical profile needed for medicinal use. This case illustrates that CEA hydroponics can dramatically boost saffron output and consistency while meeting stringent quality standards.

Mariani *et al.* (2025) have also demonstrated soilless saffron success. In Italy they grew saffron in raised wooden bins filled with organic substrate versus local farm soil and open field. Over three years, the bin-grown crops outperformed field plots: yields were 35–62% higher than controls, driven mainly by more flowers per area. Importantly, labour analysis showed that bench cultivation allowed easier planting and harvesting.

Field-scale trials in Kashmir, India also indicate promise. A 2024 report describes local farmers using small indoor hydroponic setups. Although these trials are in early stages, farmers observe that indoor growing "uses lesser water and chemicals" and protects crops from erratic weather. So far indoor yields lag behind field levels, but growers anticipate that optimization and scaling will improve results. As one farmer put it, controlled environments eliminate worries about drought or heavy rain.

2.8 Effect of plant growth regulators on morphological, floral, biochemical and quality parameters in *Crocus sativus* L.

The application of plant growth regulators (PGRs) significantly influences the growth, development, and quality traits of *Crocus sativus* L. cultivated under both soil and soilless conditions. Hormones such as auxins, gibberellins, cytokinins, and abscisic acid modulate key physiological pathways, thereby altering morphological, floral, biochemical, and spice quality parameters.

2.8.1 Effect of hormones on morphological, floral parameters and quality parameters

Morphological development in saffron (*Crocus sativus* L.) is predominantly driven by vegetative processes such as corm enlargement, daughter corm formation, root initiation, and shoot elongation. Plant growth regulators (PGRs), especially auxins, gibberellins, and cytokinins, play central roles in modulating these parameters in both soil and hydroponic systems.

Mahmoodi *et al.* (2018) applied kinetin at 20–80 $mg \cdot L^{-1}$ and found that 40 $mg \cdot L^{-1}$ was optimal for maximizing leaf area, shoot length, and chlorophyll content in field-grown saffron. Treated plants exhibited broader leaf blades and denser shoot growth, suggesting cytokinin-mediated enhancement of photosynthetic surface area and morphogenesis

Rahimi-Azar *et al.* (2019) conducted a greenhouse study where large dormant corms were soaked in IAA solutions (0–150 $mg \cdot L^{-1}$) and planted in perlite media. The 50 $mg \cdot L^{-1}$ IAA treatment yielded a 28% increase in daughter corm number and a 21% rise in total fresh weight compared to controls. Histological analysis showed activation of lateral buds due to reduced apical dominance and increased starch hydrolysis, which may serve as a carbohydrate signal for bud outgrowth.

Adhikari *et al.* (2020) focused on comparative studies from related Iridaceae (e.g., Gladiolus and Iris) and confirmed that auxin–cytokinin priming accelerated spike elongation and cormlet formation in *Gladiolus grandiflorus*,

Bhat and Kumar (2021) used indole-3-butyric acid (IBA) in a soilless ebb–flow bench system. A 1 mM IBA pre-treatment nearly doubled cormlet multiplication (mean of 9.4 per mother corm) and improved stigma yield by 15%, demonstrating that auxin analogs not only stimulate vegetative proliferation but can also enhance reproductive potential

El Fadili *et al.* (2021) reported that corms soaked in 50 ppm GA_3 exhibited enhanced shoot elongation and higher biomass accumulation. However, concentrations ≥200 ppm promoted excessive vegetative elongation, leading to reduced flower formation and depleted carbohydrate reserves in corms

Nourimand *et al.* (2022) showed that kinetin combined with blue light significantly increased SPAD chlorophyll indices and root respiration rates, correlating with increased biomass. This interaction supports the theory that cytokinin enhances source–sink relationships under optimized lighting

Patil *et al.* (2022) observed that optimal GA_3 (10–25 $mg \cdot L^{-1}$) doses lengthened floral spikes without compromising corm biomass. In conclusion, plant hormones significantly influence the morphological traits of saffron. Auxins stimulate corm proliferation and bud activation, gibberellins enhance elongation and biomass (but require precise dosing), and cytokinins promote cell division and shoot expansion. Integrative hormone treatments offer a promising strategy for improving saffron's vegetative growth in both soil-based and hydroponic cultivation systems. However, achieving a balance between vegetative and reproductive development remains crucial, necessitating continued research into dosage optimization, timing, and environmental interactions.

Morales-Garcia *et al.* (2023) performed transcriptomic profiling and found that GA_3 up regulated *CYCD 3;1* and other genes promoting G1/S cell-cycle transition, further supporting its role in shoot proliferation and leaf expansion.

Khan *et al.* (2024) demonstrated that low-dose IAA (25 $mg \cdot L^{-1}$) combined with mild cold stress (4 °C for 14 days) at sprouting induced significant up regulation of floral integrator genes (e.g., *CsFT1, CsLFY*) and promoted early bud emergence. While daughter corm yield remained stable, early morphogenesis was enhanced, showing that auxins can

initiate both vegetative and reproductive development depending on environmental context.

Lotfi and Hassani (2024) reported that IAA + kinetin (25 $mg \cdot L^{-1}$ each) improved daughter corm production by 37% over control, with significant increases in leaf area and shoot height. These results support the hypothesis that auxin–cytokinin synergy regulates apical and lateral bud differentiation via coordinated cell expansion and division pathways. Also they suggested that combined hormone applications have shown synergistic effects on morphology.

Mojtahedi *et al.* (2025) found that repeated sprays of 10 ppm GA_3 over four weeks significantly improved stigma yield (31%) and cormlet mass (14%) without causing morphological anomalies. This suggests that fine-tuning GA_3 dosage can selectively stimulate vegetative traits while preserving floral development and fractional or low-dose applications of GA_3 appear more effective.

2.8.2 Effect of hormones on biochemical parameters

The biochemical profile of saffron, particularly the synthesis of primary and secondary metabolites such as crocin, picrocrocin, flavonoids, and proline, is strongly modulated by plant growth regulators (PGRs). These biochemical markers are critical indicators of physiological health, stress responses, and spice value.

Shabani *et al.* (2018) reported that saffron corms soaked in 100 µM ABA for 24 hours exhibited a 25% reduction in DPPH IC_{50}, indicating higher antioxidant activity. Concurrently, proline levels increased by 45%, and leaf relative water content remained high under simulated drought, illustrating ABA's role in osmoprotection and ROS scavenging ABA-treated plants often redirect metabolic flux towards protective compounds, even at the expense of flowering.

Adhikari *et al.* (2020) found that auxin and cytokinin combination in *Gladiolus* increased soluble sugars and reduced starch in sprouting corms. Similarly, in *Iris hollandica*.

Li *et al.* (2020) demonstrated ABA–GA antagonism in sugar metabolism and dormancy regulation. These reinforce that saffron shares conserved biochemical hormone responses with its relatives. Biochemical parameters in saffron respond dynamically to exogenous hormone treatments. ABA boosts antioxidant compounds and proline under stress, GA_3 and auxins modulate carbohydrate partitioning, and cytokinins enhance apocarotenoid biosynthesis. The interplay between hormone concentration, application timing, and environmental conditions determines the biochemical quality of the spice.

Askari and Karimi (2020) reported that 50 µM 6-BAP upregulated *CsCCD2* and *CsUGT74AD*, key enzymes in crocin and picrocrocin biosynthesis, increasing their accumulation by 30% and 22%, respectively. This shows that cytokinins directly influence gene-level regulation of high-value compounds

Bhat and Kumar (2021) demonstrated that IBA-treated saffron corms (1 mM) grown in hydroponic systems showed elevated starch hydrolysis and a higher sucrose-to-starch ratio, leading to increased daughter corm production and stigma mass.These results support the role of auxins in modifying carbohydrate fluxes that underpin corm development.

Nourimand *et al.* (2022) demonstrated that kinetin (25 µM) under monochromatic blue light elevated crocin and picrocrocin by 34% and 26%, respectively. This was associated with upregulation of the blue-light photoreceptor gene *CsCRY1*, improved SPAD indices, and increased root respiration. Such findings suggest cytokinins may influence biochemical traits via photoreceptor and respiratory pathway enhancement.

Renau-Morata *et al.* (2021) showed that ABA at moderate doses (50 µM) for 48 hours enhanced crocin biosynthesis. Expression of *phytoene synthase* and *carotenoid cleavage dioxygenase* genes doubled, leading to a 17% increase in crocin concentration in saffron stigmas.This confirms that ABA can positively influence apocarotenoid metabolism if applied judiciously.

Moradi *et al.* (2023) combined GA_3 (50 ppm) and GABA under blue LED lighting and observed simultaneous boosts in crocin (+29%) and picrocrocin (+22%), indicating hormonal crosstalk with light and metabolite biosynthesis pathways

Khan *et al.* (2024) combined low IAA spray (25 mg·L⁻¹) with cold stress, which significantly upregulated *CsFT1* and *CsLFY*, key floral integrator genes. Biochemical analysis revealed higher soluble sugar accumulation and increased carbohydrate mobilization, contributing to more efficient floral development

Mojtahedi *et al.* (2025) aligned with findings that carbohydrate partitioning is tightly regulated by GA_3, which mobilizes reserves toward reproductive sinks without exhausting vegetative tissues.

2.8.3 Effect of hormones on quality parameters

Quality traits of saffron including the concentration of crocin, picrocrocin, safranal, and other apocarotenoids are key determinants of its commercial value and sensory profile. These secondary metabolites contribute to the spice's color, taste, and aroma, and are significantly influenced by exogenous applications of plant growth regulators (PGRs).

Kiani *et al.* (2018) assessed stored saffron from hormone-treated plants and observed that GA_3 and cytokinin applications resulted in higher crocin retention after six months of storage, as measured by UV–Vis spectroscopy and chemometric analysis. The decline in safranal content, typically associated with aging, was slower in hormone-treated samples, suggesting a preservative effect mediated through enhanced initial metabolite reserves and altered degradation kinetics.

Askari & Karimi (2020) demonstrated that 6-BAP at 50 µM upregulated genes responsible for apocarotenoid biosynthesis, such as *CsCCD2* and *CsUGT74AD*, leading to 30% and 22% increases in crocin and picrocrocin levels, respectively. In metabolomic studies, cytokinin-treated stigmas exhibited higher levels of glycosylated crocins and favorable profiles of carotenoid cleavage products. These molecular enhancements translate into deeper coloration and improved bioactivity of saffron threads.

Renau-Morata *et al.* (2021) found that 50 µM ABA applied to dormant corms increased crocin by 17% via the upregulation of *phytoene synthase* and carotenoid pathway genes Furthermore, ABA-treated plants had higher antioxidant indices, including DPPH

radical scavenging and proline levels, which contribute to improved shelf life and nutraceutical quality.

Moradi *et al.* (2023) conducted a combined treatment using 50 ppm GA_3 and gamma-aminobutyric acid (GABA) under blue LED lighting and observed enhanced crocin (+29%), picrocrocin (+22%), and flower number (+28%). The elevated apocarotenoid content was confirmed through HPLC metabolite profiling, indicating that gibberellin promotes quality by facilitating carbohydrate mobilization and secondary metabolite synthesis.

Li *et al.* (2024) used untargeted metabolomics to examine the effects of red and white supplemental LEDs combined with PGR treatments. They found that 6-BAP and GA_3 increased accumulation of trans-crocin-3, crocin-1, and kaempferol glycosides compounds linked to antioxidant activity and spice coloration. The metabolite shifts correlated with higher expression of key biosynthetic genes, reinforcing the transcriptional control exerted by PGRs on secondary metabolism

Lotfi and Hassani (2024) applied a mixture of IAA and kinetin (25 $mg \cdot L^{-1}$ each), which resulted in a 37% increase in daughter corm yield while maintaining high crocin content and favorable sensory indices. Metabolomic assays confirmed elevated levels of crocin isomers (trans-crocin-4, crocin-2) and flavonoids in treated samples, revealing that such combinations optimize both biomass and biochemical outcomes. In conclusion, the application of plant hormones either singly or in combination profoundly impacts the quality attributes of saffron. Through gene expression modulation, enhanced metabolite biosynthesis, and improvements in post-harvest stability, PGRs such as cytokinins, GA_3, IAA, and ABA offer a potent tool for improving the sensory and biochemical excellence of saffron. Future directions should include integrated metabolomic and transcriptomic analyses across multiple growth stages and environmental conditions to further refine hormone-based quality enhancement strategies.

2.9 Nutrient and hoagland solution management for saffron and related bulbous crops under hydroponic conditions

The successful hydroponic cultivation of saffron (*Crocus sativus* L.), a high-value, sterile geophyte of the Iridaceae family, depends significantly on the optimization of

nutrient solutions. Saffron's growth and productivity are governed by precise balances of macro- and micronutrients, with solution formulation and electrical conductivity (EC) being critical for maximizing stigma yield and cormlet proliferation. Recent studies have also extended nutrient research to related bulbous crops in the Iridaceae and other families, offering comparative insights that guide best practices for soilless systems.

2.9.1 Macronutrient management in hydroponic saffron

Chitarra *et al.* (2016) concluded in their studies that calcium and magnesium are crucial for structural integrity and chlorophyll synthesis. Optimal ranges were found to be 60–100 mg·L⁻1 Ca and 30–50 mg·L⁻1 Mg, with high Ca:Mg ratios potentially reducing Mg uptake.

Papadakis *et al.* (2019) observed that increasing EC from 2.0 to 3.0 dS·m⁻1 enhanced K+, Ca^{2+}, and Mg^{2+} uptake, leading to larger daughter corms. However, beyond 3.0 dS·m⁻1, osmotic stress inhibited flowering. The study concluded that potassium uptake correlated most closely with stigma yield, highlighting the critical role of K in reproductive development.

Dewir *et al.* (2022) Macronutrients including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are essential for saffron's vegetative development and stigma production. They used different combinations of NPK ratios in ebb-and-flow systems and found that saffron corms exhibited optimal growth when supplied with 163 mg·L⁻1 N, 34 mg·L⁻1 P, and 173 mg·L⁻1 K at an EC of 1.4 dS·m⁻1 and pH 5.8. Excess nitrogen levels (>200 mg·L⁻1) promoted vegetative growth at the expense of flowering, reducing crocin content in stigmas.

Yadav *et al.* (2022) demonstrated that a higher NO_3^-:NH_4^+ ratio (5:1) improved vegetative growth and chlorophyll content while maintaining a balanced osmotic environment. This adaptation of macronutrient form aligns with findings in *Iris ensata* and *Narcissus*, where ammonium-dominant feeds were associated with root stress and chlorosis.

Singh *et al.* (2023) evaluated a nano-NPK formulation in saffron grown under nutrient film technique (NFT) and observed a 22% increase in daughter corm weight and

an 18% improvement in stigma yield compared to conventional soluble NPK. Nanoencapsulation allowed for prolonged nutrient availability, reducing leaching and enhancing uptake efficiency. Similar improvements were reported with controlled-release formulations in *Allium cepa* and *Lilium* spp., suggesting cross-applicability to other bulbous crops.

2.9.2 Micronutrient influence on yield and biochemical quality

Salas *et al.* (2020) recommended micronutrient formulation typically using chelated Fe-EDTA, $MnSO_4$, $ZnSO_4$, $CuSO_4$, H_3BO_3, and Na_2MoO_4 was recommended by for balanced growth and consistent crocin levels.

Ali and Shafiq (2021) reported that foliar B application at 0.3 $mg \cdot L^{-}1$ increased stigma yield by 40%, suggesting boron's critical role in sugar transport and stigma development. Zinc and manganese supported auxin biosynthesis and photosystem II function, while Mo aided nitrate assimilation.

Sheha (2021) found that trace elements such as iron (Fe), boron (B), manganese (Mn), zinc (Zn), copper (Cu), and molybdenum (Mo) are equally important in saffron's hydroponic systems. She also showed that Fe deficiency led to chlorosis and flower abortion, while optimal levels (1.5–2.0 $mg \cdot L^{-}1$ Fe-EDTA) improved chlorophyll and flower production.

Kumar *et al.* (2022) showed that Mn-deficient lilies produced significantly fewer scapes and experienced chlorotic leaf margins, underscoring the importance of micronutrient calibration even in ornamental bulb crops. Comparative studies in *Lilium longiflorum* and *Hyacinthus orientalis* revealed that Mn and Zn directly regulate reactive oxygen species detoxification and flavonoid biosynthesis.

Patel *et al.* (2023) found that supplemental Si (50 ppm sodium silicate) in saffron enhanced antioxidant enzyme activities, including SOD and CAT, while maintaining higher relative water content and crocin stability under drought simulation. Although not a traditional micronutrient, Si supplementation may be particularly relevant in recirculated hydroponic systems where stress induction from EC fluctuations is common.

2.9.3 Hoagland and modified nutrient solutions in saffron

Garcia-Navarro & Hernandez (2017**)** concluded that maintaining pH between 5.5–6.0 was essential to ensure micronutrient bioavailability, and an EC of ~2.0 $dS \cdot m^{-1}$ optimized stigma yield and daughter corm size. Regular monitoring and recirculation helped minimize nutrient waste and ensured consistency in root-zone availability.

Adhikari *et al.* (2020) Nutrient management strategies from related Iridaceae crops support and extend saffron findings. In *Gladiolus*, demonstrated that balanced NPK (150:60:150 $mg \cdot L^{-1}$) improved floret emergence and spike elongation under hydroponics. In *Iris hollandica*,

Li *et al.* (2020) observed that nitrate-dominant solutions improved dormancy break and flowering synchrony.

Wang *et al.* (2020) In *Freesia hybrida* studies indicated that elevated Fe and B levels improved pigment synthesis and bulb mass these analogs reinforce the role of tailored micronutrient blends and EC–pH management in enhancing performance across geophytic systems.

Dewir *et al.* (2022) The Hoagland solution is widely used in hydroponic research due to its balanced profile and modified the Hoagland formulation to accommodate saffron's moderate nutrient demands. A representative composition included:

- N: 150–180 $mg \cdot L^{-1}$ (3:1 NO_3^-:NH_4^+)
- P: 40–60 $mg \cdot L^{-1}$
- K: 175–200 $mg \cdot L^{-1}$
- Ca: 100–120 $mg \cdot L^{-1}$
- Mg: 30–50 $mg \cdot L^{-1}$
- Micronutrients as per standard Hoagland trace mix

CHAPTER 3

MATERIALS AND METHODS

The present study entitled "Studies of saffron (*Crocus sativus* L.) cultivated under soil and soilless growing conditions" was designed to compare the performance *of Crocus sativus* L. cultivated under traditional soil-based and soilless (hydroponic) systems across three distinct agro-ecological regions of Jammu & Kashmir. The Field trials were conducted during the year 2022 & 2023 growing seasons at two mountainous locations i.e. Kashmir valley and Kishtwar where saffron was grown in native loamy–silty soils. In contrast, a controlled soilless (hydroponic) cultivation system was established at the experimental glasshouse was carried out in the Department of Plant Physiology, Faculty of Basic Sciences, Sher-e- Kashmir University of Agricultural Science & Technology of Jammu, Main Campus Chatha, during the year 2022 & 2023.

3. Description of study area

3.1 Experimental sites and climatic conditions

To capture the influence of both natural and controlled environments on saffron performance, three distinct locations were selected for study: (i) a traditional soil-based field in the Kashmir valley, (ii) a soil-based field in the Kishtwar region, and (iii) a soilless (hydroponic) system in a controlled glasshouse at SKUAST- Jammu. The geographic, edaphic and meteorological characteristics of each site are summarized below.

3.1.1 Kashmir valley field site

Location and altitude: 34°06′N, 74°47′E; 1,600–1,800 m above sea level (ASL) as shown in Plate 1.

Soil characteristics: Native alluvial loamy–silty soil with good drainage; average pH 6.8; organic matter ~3.5 %; field capacity ~28 %; texture class Loam.

Climate

- Mean annual temperature: 10–14 °C (summer max up to 25 °C; winter min down to –5 °C).

- Average annual rainfall: ~650 mm, predominantly June–September.
- Relative humidity: 65–75 % (highest during spring).
- Photoperiod: 10–14 h of daylight (depending on season).

3.1.2 Kishtwar field site

Location and altitude: 33°18′N, 75°46′E; 1,200–1,400 m (ASL) as shown in Plate 2.

Soil characteristics: Silty–loam soil derived from greywacke; pH 6.5; organic matter ~2.8 %; field capacity ~30 %; good aeration.

Climate:

- Mean annual temperature: 12–16 °C (summer peaks ~30 °C; winter lows ~0 °C).
- Average annual rainfall: ~800 mm, with monsoon contributing ~60 %.
- Relative humidity: 60–70 %, with high morning dew.
- Photoperiod: 10–14 h daylight through the growing season.

3.1.3 Jammu – SKUAST

Location and altitude: SKUAST Jammu Glasshouse, 32°44′N, 74°52′E; ~300 m (ASL) as shown in Plate 3.

System setup: Nutrient Film Technique (NFT) channels housed under polycarbonate panels.

Environmental control

- **Temperature:** Maintained at 20 ± 2 °C (day), 16 ± 2 °C (night).
- **Relative humidity:** 60–70 %.
- **Photoperiod:** Supplemental lighting to ensure 14 h light/10 h dark during low-light months.

Plate 1 Geospatial location of Pampore, the traditional saffron cultivation hub of Kashmir

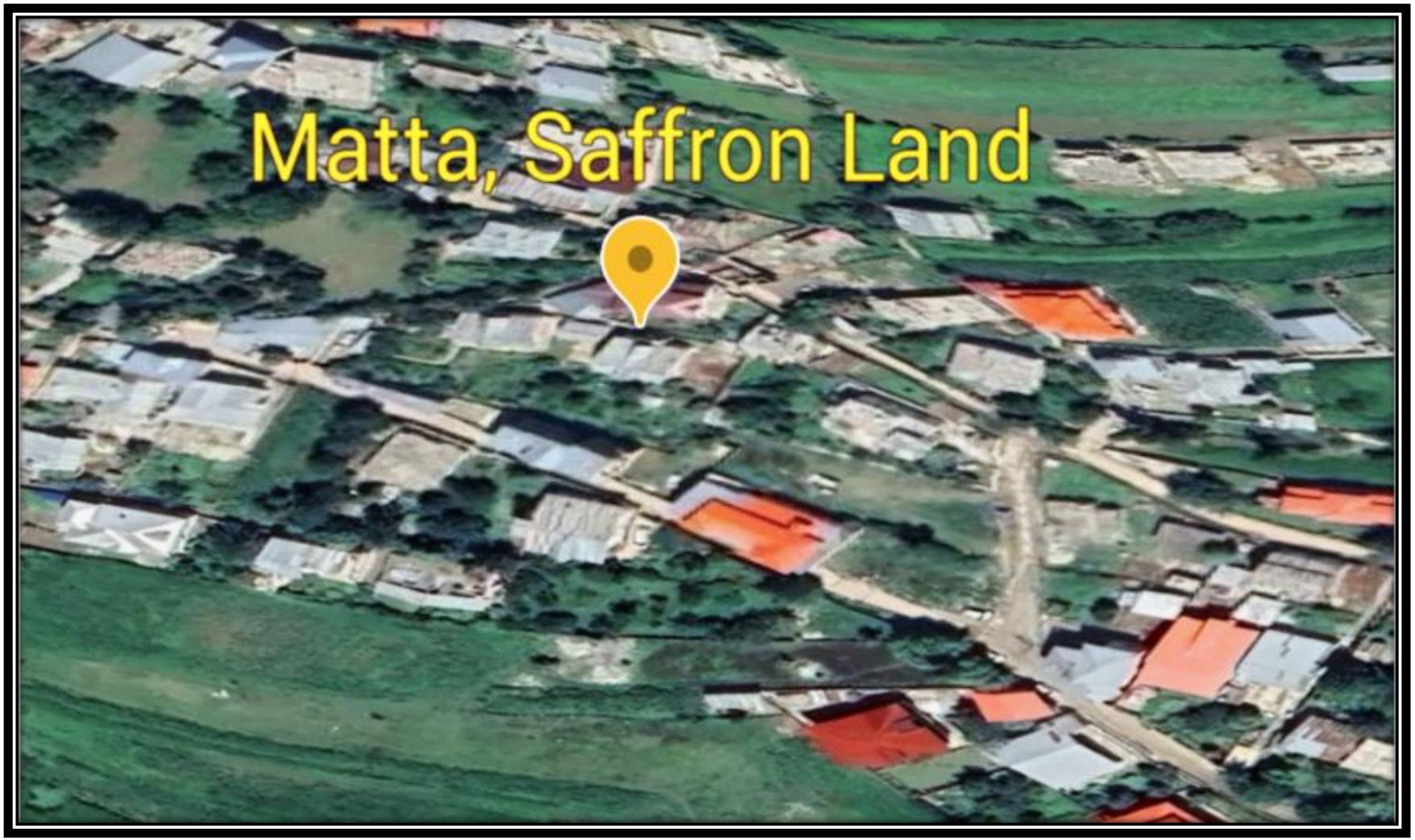

Plate 2 Geospatial view of Matta village, an emerging saffron cultivation site in Kishtwar

Plate 3 Geospatial view of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J)

a. Collection of healthy corms

b. Tradition method used for sowing corm

c. Knowledge exchange on indigenous saffron farming

d. Emergrence of saffron (*Crocus sativus*) flowers in Pampore

Plate 4 Conventional study on production of saffron (*Crocus sativus*) in Pampore, (Kashmir valley)

a. Preparation of land

b. Sowing of corms

c. Application of fertilizers in the soil

d. Emergence of saffron flower (*Crocus sativus*) in Kishtwar

Plate 5 Conventional study on production of saffron (*Crocus sativus*) in Matta village (Kishtwar)

By selecting these three environments i.e. two representating high-altitude, low-altitude (soil-based fields) and soilless system (controlled conditions), this study rigorously evaluates how edaphic and climatic variables influence the morpho-physiological development and biochemical quality of *Crocus sativus* L.

3.2 Date of corm sowing

Locations	Method	Date
Kashmir	Conventional	21st of September, 2022
Kishtwar	Conventional	15th of October, 2023
Jammu (Ist trail)	Hydroponics	8th of August, 2022
Jammu (2nd trail)	Hydroponics	10th of September, 2023

Variety: SRS-Saf-175

Replications: 05

3.3 Materials and methods

3.3.1 Plant material and preparation

The following steps were undertaken to source, select, and pre-treat saffron corms for both soil-based (Kashmir and Kishtwar) and hydroponic (Jammu) experiments. The soil-based trials served as the untreated control, whereas the hydroponic corms received hormone and nutrient-solution treatments.

1. Sourcing of corms

- Uniform, disease-free corms of *Crocus sativus* L. (8–10 g weight; 3–4 cm diameter) were procured from a certified saffron grower in Pampore, Kashmir.
- Corms were transported to the laboratory within 24 h, packed in ventilated crates to avoid moisture accumulation.

2. **Initial cleaning and sorting**

   - Upon arrival, corms were gently brushed to remove adhering soil and debris.
   - Damaged, diseased, or misshapen corms were discarded; only firm, intact corms with a smooth tunic were retained.

3. **Surface disinfection**

   - Selected corms were submerged in a 0.1% (v/v) sodium hypochlorite solution for 5 minutes to eliminate surface pathogens.
   - They were then rinsed three times with sterile distilled water to remove any residual bleach.

4. **Drying and resting**

   - Disinfected corms were placed on sterile trays under a laminar-airflow hood for 2 hours to air-dry their surface moisture.
   - Afterwards, corms were stored at 10 ± 2 °C and 65 ± 5% RH for 48 hours to stabilize before treatment.

5. **Corm soaking for hormone treatments**

   - Each treatment group was immersed in its respective hormone solution for 8 hours at 15 ± 2 °C in darkness.
   - The nutrient-solution group was soaked similarly in Hoagland's solution without hormones.
   - After soaking, corms were briefly rinsed with sterile distilled water to remove excess solution.

6. **Transfer to hydroponic system:** Only uniformly sized (3–4 cm diameter), disease-free corms that had soaked in their respective hormone solutions (IAA, GA_3, kinetin or ABA) for 8 h and exhibited no surface damage were chosen for transplanting. (as shown in plate 6 and 7)

(a) Collection of corms

(b) Cleaning of healthy corms

(c) Corms dipped in hormonal solution

Plate 6 Collection, cleaning, and application of hormonal solution to corms for hydroponic study in SKUAST-Jammu

(a) Vermicompost

(b) Coco peat

(c) Vermiculite

(d) Sowing saffron corms in hydroponic cups

Plate 7 Preparation and sowing of corms with substrates in hydroponic cups

3.4 Designation of treatment groups

Treatment	Method & Location	Treatment Combinations
T_1	Hydroponic (Jammu)	(1 ppm Indole-3- Acetic Acid) + Nutrient solution
T_2	Hydroponic (Jammu)	(1 ppm Kinetin)+ Nutrient solution
T_3	Hydroponic (Jammu)	(50 ppm GA_3) + Nutrient solution
T_4	Hydroponic (Jammu)	(1.5 ppm ABA) + Nutrient solution
T_5	Conventional (Kashmir)	(1:1) Soil + Vermicompost (No PGRs)
T_6	Conventional (Kishtwar)	(1:1) Soil + Vermicompost (No PGRs)

3.5 Hormone treatments used in hydroponic system

1. Reagents and equipment for preparation of hormone solution

- **Hormones:** Indole-3-acetic acid (IAA, ≥ 99%); Gibberellic acid (GA_3, ≥ 95%); Kinetin (6-furfurylaminopurine, ≥ 98%); Abscisic acid (ABA, ≥ 98%). All from Sigma-Aldrich.
- **Solvents:** 1 N NaOH; 95% ethanol; dimethyl sulfoxide (DMSO).
- **Glassware:** 1 L and 100 ml volumetric flasks; amber reagent bottles; analytical balance (± 0.1 mg); pipettes.

2. Stock solution preparation (ppm)

1. IAA (1 000 ppm)

- 1 000 mg IAA was weighed and transferred into a 1 L volumetric flask.
- ~5 ml of 1 N NaOH was added; the flask was swirled until the IAA fully dissolved.

- The volume was made up to 1 000 ml with distilled water and pH was adjusted to 6.0 with 1 N HCl.

2. **GA_3 (1 000 ppm):**
   - 1 000 mg GA_3 was placed in a 1 L flask with ~5 ml 95% ethanol.
   - The mixture was warmed in a 40 °C water bath for 10 min until clear, cooled, then diluted to 1 L with distilled water.
3. **Kinetin (1 000 ppm):**
   - 1 000 mg kinetin was transferred to a 1 L flask and dissolved in ~5 ml DMSO by gentle swirling.
   - After complete dissolution, the volume was brought to 1 000 ml with distilled water.
4. **ABA (1 000 ppm):**
   - 1 000 mg ABA was added to a 1 L flask with 10 ml 95% ethanol.
   - The flask was placed in a 40 °C water bath until the ABA dissolved, cooled, then filled to 1 L with distilled water.

Storage: *All stock solutions were kept in amber bottles at 4 °C and used within four weeks.*

3. Working solution preparation

On the day of treatment, aliquots of each stock were diluted to 1 L to yield the target concentrations:

Hormone	Stock concentration	Aliquot taken	Final volume	Final concentration
1. IAA	1 000 mg L^{-1}	1.0 ml	1 000 ml	1 mg L^{-1} (1 ppm)
2. GA_3	1 000 mg L^{-1}	1.0 ml	1 000 ml	1 mg L^{-1} (1 ppm)
3. Kinetin	1 000 mg L^{-1}	50.0 ml	1 000 ml	50 mg L^{-1} (50 ppm)
4. ABA	1 000 mg L^{-1}	1.5 ml	1 000 ml	1.5 mg L^{-1} (1.5 ppm)

Table 3.1 Composition of the nutrient solution used in the hydroponic experiment (g L^{-1})

S. No.	Nutrient	g L^{-1}
1.	Sulfur (S)	2.0 g L^{-1}
2.	$MgSO_4 \cdot 7H_2O$	5.0 g L^{-1}
3.	$CaCl_2 \cdot 2H_2O$	5.0 g L^{-1}
4.	NaCl	50.0 g L^{-1}
5.	H_3BO_3	0.2 g L^{-1}
6.	Fe-EDTA (Fe equiv.)	0.2 g L^{-1}
7.	$MnCl_2 \cdot 4H_2O$	0.01 g L^{-1}
8.	$CuSO_4 \cdot 5H_2O$	0.01 g L^{-1}
9.	$ZnSO_4 \cdot 7H_2O$	0.05 g L^{-1}

3.6 Nutrient solution preparation

3.6.1 Materials and equipment

S.No.	Chemicals	Analytical grade
1.	Sulfur	Powder
2.	Magnesium sulfate heptahydrate	($MgSO_4 \cdot 7H_2O$)
3.	Calcium chloride dihydrate	($CaCl_2 \cdot 2H_2O$)
4.	Sodium chloride	(NaCl)
5.	Boric acid	(H_3BO_3)
6.	Iron–EDTA complex	(Fe-EDTA)
7.	Manganese chloride tetrahydrate	($MnCl_2 \cdot 4H_2O$)
8.	Copper sulfate pentahydrate	($CuSO_4 \cdot 5H_2O$)
9.	Zinc sulfate heptahydrate	($ZnSO_4 \cdot 7H_2O$)

Laboratory apparatus: Analytical balance (±0.1 mg), volumetric flasks (100 ml, 1 L), graduated cylinders, magnetic stirrer with stir bars, pH meter with calibration buffers

(pH 4.0, 7.0, 10.0), electrical conductivity (EC) meter, syringe filters (0.22 µm) or vacuum-filtration setup, amber polypropylene bottles (500 ml) for stock storage and deionized water.

3.6.2 Stock solution preparation

3.6.2.1 Macronutrient stock (100× concentrate; prepare 1 L)

1. In a 1 L volumetric flask, add approximately 800 ml of deionized water.
2. Weigh and dissolve, stirring with a magnetic stirrer each of the following to yield a 100× stock of the final working concentrations:

1.	Sulfur powder	200 g
2.	$MgSO_4 \cdot 7H_2O$	500 g
3.	$CaCl_2 \cdot 2H_2O$	500 g
4.	NaCl	50 g

3. After complete dissolution, dilute to the 1 L mark with deionized water.
4. Filter through a 0.22 µm syringe filter and transfer to a labelled amber bottle: "Macronutrient Stock (100×)". Store at room temperature.

3.6.2.2 Micronutrient stock (1,000× concentrate; prepare 1 L)

5. In a separate 1 L volumetric flask, add ~800 ml deionized water.
6. Weigh and dissolve

1.	H_3BO_3	0.2 g
2.	Fe-EDTA	0.2 g
3.	$MnCl_2 \cdot 4H_2O$	0.01 g
4.	$CuSO_4 \cdot 5H_2O$	0.01 g
5.	$ZnSO_4 \cdot 7H_2O$	0.05 g

7. Bring volume up to 1 L with deionized water, mix until clear.

8. Filter as above and label "Micronutrient Stock (1,000×**)"**. Store at 4 °C.

3.6.3 Working solution preparation

Formulation (per 1 L)	
Macronutrient stock (100×)	10 ml
Micronutrient stock (1,000×)	1 ml
Dionised water	1 L

3.6.4 Adjustment and quality check

- **EC:** Adjusted to 1.5 dS m^{-1} by dilution or small additions of macronutrient stock.
- **pH:** Adjusted to 5.8–6.2 using 0.1 M HCl or 0.1 M KOH.
- Stir for 10 min and re-verify EC and pH.

Usage in NFT system

- Replaced the nutrient solution in the hydroponic channels weekly.
- Discarded remaining working solution after one week;
- Prepared fresh solution to avoid microbial growth and nutrient precipitation.

All preparation steps were carried out under clean-room conditions to minimize contamination. Stock solutions were checked monthly for precipitate formation, and replaced if turbidity was observed.

Conventional trials in Kashmir & Kishtwar: Corms allocated to the soil-based experiments were soaked in sterile distilled water for 8 hours and then planted directly as shown in Plate 4 and 5).

3.7 System design and construction

1. Frame and channels

- A frame (2.5 m × 1.0 m × 1.0 m) was assembled to support six parallel PVC channels (3 m length × 100 mm width × 50 mm depth).
- Each channel was inclined at 1.5° to allow a continuous thin film of nutrient solution to flow over the corm basal plates.

2. Reservoir and pump

- A 200 L opaque polyethylene reservoir collected the recirculating solution.
- A submersible pump (300 L h^{-1}) delivered solution via 6 mm tubing to the uphill end of each channel.
- Return flow was gravity-fed back into the reservoir.

3. Corm supports

- Coco peat (20%), Vermiculite (10%), Vermicompost (60%), and Perlite (10%), which provides a balanced mixture of moisture retention, aeration, and nutrient availability.

3.8 Experimental layout

Randomization

- Each treatment (IAA, GA_3, kinetin, ABA,) was assigned to two channels (n = 50 corms per treatment; total 200 corms).
- Channels were arranged side by side, treatments were randomized across the frame to minimize positional bias.

3.9 Environmental control and monitoring

- **Climate Conditions:** The glass house was maintained at 18 ± 2 °C (night) / 24 ± 2 °C (day), 65 ± 5% RH, under a 12 h photoperiod (400 µmol m^{-2} s^{-1} via LED lamps).

- **Solution parameters:** pH and EC were measured twice daily (morning and evening) and adjusted as necessary.
- Successful flowering was observed after 13 weeks of corm treatments.

3.10 Observations

The following observations were recorded at seedling, vegetative and reproductive stages as shown in Plate 7, 8 & 9.

3.11.1 Morphological parameters

a) Number of leaves per plant: The total fully expanded leaves on each plant were counted by hand.

b) Plant height (cm): Height was measured from the substrate surface to the tip of the longest leaf using a metric ruler.

c) Total chlorophyll (SPAD): Readings were taken on the middle portion of the newest fully expanded leaf; six readings per leaf were averaged.

d) Fresh weight of leaves (g): All leaves were excised at the collar, gently blotted on filter paper, and immediately weighed on a digital balance (± 0.01 g).

e) Dry weight of leaves (g): The same leaf samples were oven-dried at 70 °C for 72 h to constant weight and then re-weighed.

3.11.2 Floral attributes

a) Weight of flower (g): Entire flowers were harvested, blotted, and weighed fresh on a precision balance.

b) Pistil length (cm): Pistils were carefully separated, laid flat, and measured from base to tip using a digital caliper (± 0.01 cm).

c) Fresh weight of pistil (g): Pistils were immediately weighed after excision.

d) Dry weight of pistil (g): Pistils were dried at 60 °C for 48 h before being weighed.

e) **Number of flowers per corm:** The total flowers produced by each corm over the 40 days were counted.

f) **Stigma length (cm):** Stigmas were measured from base to tip with a digital caliper.

g) **Fresh weight of stigma (g):** Stigmas were pooled by corm, blotted, and weighed.

h) **Dry weight of stigma (g):** Stigmas were oven-dried at 60 °C for 48 h and then weighed.

3.11.3 Corm attributes

a) **Number of daughter corms/mother corm:** At the end of the experiment, corm clusters were carefully excavated and daughter corms were counted per original mother corm.

b) **Average weight of daughter corms (g):** Individual daughter corms were cleaned, blotted, and weighed fresh; the mean weight per mother corm was calculated.

3.11.4 Biochemical and quality parameters

3.11.4.1 Spectrophotometric analysis of crocin, crocetin, picrocrocin and safranal content at different wavelengths Bathaie, S. Z. *et al*., 2007

1. Materials and reagents

- **Saffron sample** (dried stigmas), finely powdered.
- **Solvents:** ethanol and deionized water.
- **Extraction solvent:** 80% (v/v) ethanol in water.
- **Equipment:** UV-Vis spectrophotometer (200–700 nm range) with 1 cm quartz cuvettes, analytical balance (±0.1 mg), mortar and pestle, ultrasonic bath or shaker, centrifuge capable of 10 000 × g, volumetric flasks (10, 25, 50 ml), micropipettes and pipette tips 0.45 µm syringe filters.

Initiation of sprouting of corms

Initiation of leaf primordia and shoot tip

Plate 8 Emergence of leaf primordia and shoot tips after 10 days of application of treatment

Plate 9 Flowering of *Crocus sativus* under nutrient film technique

After dissection

Plate 10 **Manual collection and post-harvest handling of stigmas from hydroponically grown *Crocus sativus***

2. **Sample preparation**

   a. **Weighing:** 100 mg of powdered saffron was accurately weighed and transferred into a 10 ml volumetric flask.

   b. **First extraction:**

   - 10 ml of 80% ethanol was added.
   - The flask was sonicated (or shaken) for 30 minutes at room temperature, in the dark.

   c. **Centrifugation:**

   - The mixture was centrifuged at 10 000 × g for 10 minutes.
   - The supernatant was collected; the residue was re-extracted with another 10 ml of 80% ethanol.

   d. **Pooling and clarification:**

   - Supernatants were combined in a 25 ml volumetric flask and brought to volume with 80% ethanol.
   - The combined extract was filtered through a 0.45 µm membrane to remove particulates.

3. **Spectral measurement**

   a) **Blank:** A cuvette was filled with 80% ethanol and used to zero the spectrophotometer.

   b) **Recording absorbance:** Aliquots of the clarified extract were transferred to quartz cuvettes. Absorbance was measured at the following λ max:

   - **Picrocrocin** at 257 nm
   - **Safranal** at 330 nm

- **Crocin** at 440 nm
- **Crocetin** at 430 nm

c) Calculations: Using Beer–Lambert's law,

$$A=\varepsilon cl$$

Where, A = the absorbance at the compound's λ max,

ε = the molar extinction coefficient (L mol^{-1} cm^{-1}),

c = the molar concentration (mol L^{-1}), l is the path length (cm, here 1 cm).

Rearranged for concentration,

$$C_{mg/g}=\frac{AxVxMW}{\varepsilon xLxm}\times 10^3$$

Where, V = the final extract volume (L),

MW = the molecular weight of the analyte (g mol^{-1}), and m is the mass of saffron used (g).

3.11.4.2 Proline content

Proline content was estimated by using the modified method of Bates *et al*., 1973.

Reagents

(i) 3 % aqueous sulphosalicylic acid (w/v)

(ii) Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M o-phosphoric acid until dissolved)

(iii) Toluene

Extraction

Fresh leaves, weighing 300 mg each, were separately homogenized in 5 ml of 3% sulphosalicylic acid, followed by centrifugation at 5000 rpm for 15 minutes, after which the supernatant was collected.

Procedure

Two milliliter of the supernatant was placed in a test tube, to which 2.0 ml of acid ninhydrin reagent was added. The mixture was then heated in a boiling water bath at 100°C for 1 hour, after which the reaction was halted by transferring the test tubes to an ice bath. Following this, 4.0 ml of toluene was added and the mixture was shaken vigorously. Once it reached room temperature, the upper coloured organic layer was extracted and its absorbance was measured at 520 nm using toluene as a blank. A standard curve was generated with known concentrations of proline in 3% sulphosalicylic acid and proline content was expressed as μmol g^{-1} FW.

3.11.4.3 DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed following the protocol established by Abe *et al*., 1998. The sample was prepared at a 1:10 ratio (1 g of powdered grain sample in 10 ml of methanol) and left on a shaker overnight. The solution was subsequently filtered using filter paper. Aliquots of 1 ml of 0.5 mM DPPH methanol solution were combined with 100 µl, 200 µl, 300 µl and 400 µl of the sample, followed by the addition of 2 ml of 0.1 M sodium acetate buffer (pH 5.5). This reaction mixture was agitated and allowed to incubate at room temperature in darkness for 30 minutes. Absorbance was recorded at 517 nm with a double-beam UV-VIS spectrophotometer, using methanol as the negative control. The radical scavenging activity (RSA) was calculated as the percentage of DPPH radical decolorization using the following equation:

$$\text{Scavenging effect (\%)} = \frac{1 - \text{A sample}}{\text{A blank}} \text{ X } 100$$

Where A sample represents the absorbance of the test sample and A blank represents the absorbance of the control containing all reagents except the test sample.

By plotting the percentage inhibition of DPPH radical scavenging activity against extract concentration, the extract concentration that produces 50% inhibition (IC_{50}) was determined. Butylated hydroxytoluene (BHT) was used as a reference standard.

3.11.4.4 Phenolic content

The polyphenols contained in plant extracts are usually assessed by the Folin-Ciocalteu assay, the method developed by (Singleton and Rossi 1965). In the case of hydrophilic extracts, the following protocol was carried out: to 100 L of each hydrophilic extract at the different pH's were added 900 L of distilled H_2O, 50 L of 1 M sodium carbonate, 50 L of Folin Ciocalteu's reagent, and finally All was diluted with 2 mL of distilled H_2O. In contrast, the extracts in the lipophilic phase were brought to dryness in a Heildolph Laborota 4001 rotary evaporator (Schwabach, Germany) with a 40 C bath and a Büchi V-700 (Barcelona, Spain) vacuum pump (240mbar) connected to a VWRRC-10 (Barcelona, Spain) digital chiller to evaporate the solvent. The dried residue was resuspended with 600 L of 70% methanol. Lastly, 200 L of the resuspended extracts were taken with a pipette and 800 L of distilled water were added, followed by the addition of the other reagents used to determine the amount of phenol in the same way and in the same quantities. All reaction mixtures were kept in a water bath at 30 C for 15 min and then it was measured at 755 nm. The total phenol concentration was expressed as moles of gallic acid equivalents (GE) per gram dry weight (moles GE/100 g DW).

3.11.4.5 Flavonoid content

The aluminium chloride colorimetric method, modified by Woisky and Salatino (1998) was used to determine the flavonoid content. On the other hand, extracts brought to dryness were used to measure lipophilic extracts. To 500 L of the extract, 1.5 ml of 96% ethanol was added. The other reagents were added in the same way and amount as for the hydrophilic extracts until a final volume of 5 ml was reached. The mixtures were kept in the dark for 30 min and then the optical density was measured at 415 nm (ElMihyaoui, A *et. al*., 2021). Quercetin (QE), a flavanol found as an O-glycoside in high concentrations in both fruits and vegetables, was used as a standard. A calibration curve was obtained. The total flavonoids were expressed as moles of quercetin equivalents per gram dry weight (moles QE/g DW). To express the results, all replicates were averaged. The standard error and relative error were calculated

Statistical analysis

Data were analysed using completely randomized design (CRD) for two factors. Treatments were compared using critical difference (CD) at 5 % level of significance. Data were subjected to analysis of variance (ANOVA) using R software.

CHAPTER 4

RESULTS

This chapter presents the experimental findings derived from comparative evaluations of *Crocus sativus* L. cultivated under conventional soil-based systems in Kashmir and Kishtwar, and hydroponic setups in Jammu. The observations encompass a wide spectrum of morpho-physiological, floral, corm-related, biochemical, and antioxidant parameters assessed across different treatment combinations involving plant growth regulators (IAA, kinetin, GA_3, and ABA). Results are organized systematically to highlight variations induced by cultivation methods, hormonal applications, and environmental conditions. Significant effects on plant height, number of leaves, stigma yield, corm development, pigment concentrations, and antioxidant potential are analyzed and interpreted in the context of crop performance and quality. The data are presented in tabular and graphical formats, providing critical insights into the adaptive response of saffron to soil and soilless environments under varying agro-climatic regimes.

4.1 Morphological parameter

4.1.1 No. of leaves per plant

A significant variation in the number of leaves per plant (Table 4.1 and Fig. 4.1) was observed among treatments. The highest counts were recorded in the treatment T_5 (22.17 and 23.00 leaves/plant), followed by treatment T_2 i.e. kinetin @ 1 ppm (21.00) and Treatment T_3 GA_3 @ 50 ppm (19.33), whereas treatments T_1 and T_4 i.e. IAA @ 1 ppm and ABA @ 1.5 ppm produced notably lower leaf numbers of 13.67 and 5.67, respectively, with respect to the treatment T_5 and T_6 (22.17 and 23.00). However, differences among the Kashmir and Kishtwar (conventional farming), kinetin and GA treatments were non-significant, while the ABA treatment exhibited a significantly reduced number of leaves per plant (CD = 2.90).

Table 4.1 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of leaves per plant of *Crocus sativus*.

Method & Location	Treatments	No of leaves/ plant
Hydroponics (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	13.67
Hydroponics (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	21.00
Hydroponics (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	19.33
Hydroponics (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	5.67
Conventional (Kashmir)	T_5 (No PGRs)	22.17
Conventional (Kishtwar)	T_6 (No PGRs)	23.00
CD (p≤0.05)		**2.90**

4.1.2 Plant height

Plant height varied significantly across treatments (Table 4.2 and Fig. 4.2), with the tallest plants in the treatment T_5 and T_6 i.e. Kashmir and Kishtwar, where used conventional method (24.88 and 27.13 cm), followed by treatment T_3 i.e. GA3 @ 50 ppm (23.74 cm), whereas treatment T_1 and T_2 i.e. IAA @ 1 ppm and kinetin @ 1 ppm yielded intermediate heights of 18.11 cm and 16.02 cm, respectively, and treatment T_4 i.e. ABA @ 1.5 ppm resulted in the shortest plants at 7.63 cm with respect to the Conventional farming i.e. treatment T_5 and T_6 (24.88 and 27.13). However, differences between the conventional methods used in Kashmir and Kishtwar, GA3 treatment were non-significant, while the ABA treatment led to a significant reduction in plant height (CD = 3.35).

Table 4.2 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on plant height (cm) of *Crocus sativus*.

Method & Location	Treatments	Plant height (cm)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	18.11
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	16.02
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	23.74
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	7.63
Conventional (Kashmir)	T_5 (No PGRs)	24.88
Conventional (Kishtwar)	T_6 (No PGRs)	27.13
CD (p≤0.05)		**3.35**

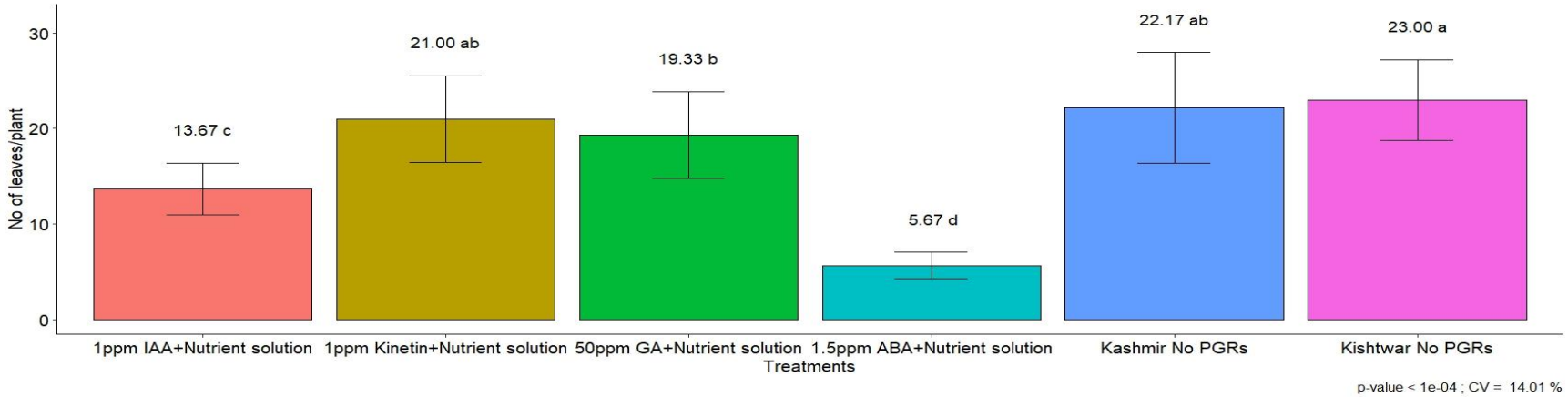

Fig. 4.1 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of leaves per plant of *Crocus sativus*

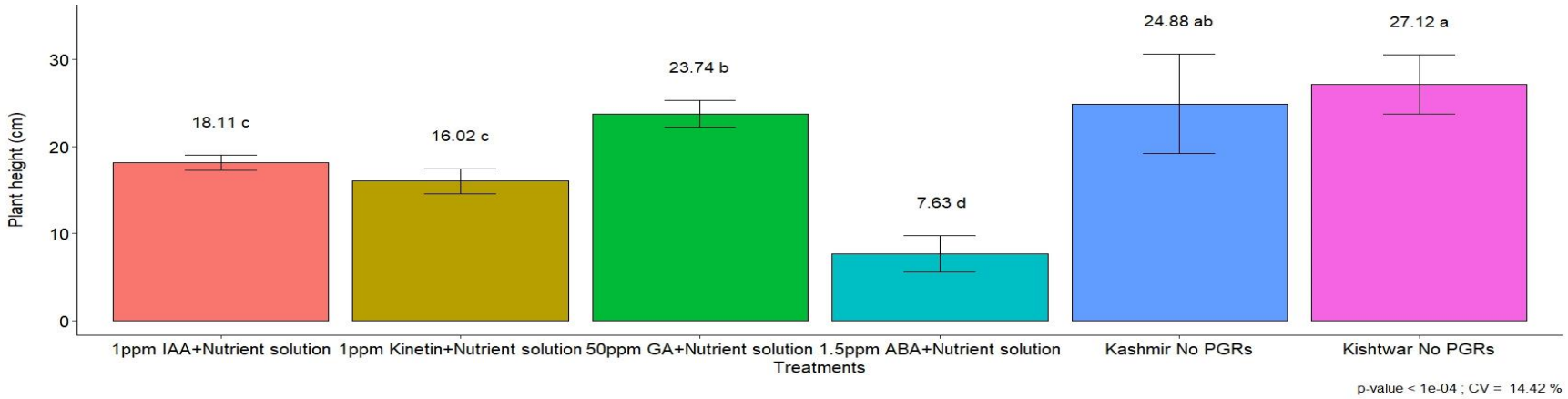

Fig. 4.2 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on plant height (cm) of *Crocus sativus*

4.1.3 Leaf chlorophyll content

As estimated by SPAD values, differed significantly among treatments as depicted in Table 4.3. SPAD values ranged from a low of 30.61 in plants treated with IAA @ 1 ppm (T_1) to a high of 56.24 in the conventional Kishtwar control (T_6). The GA_3-treated hydroponic plants (T_3) exhibited the second-highest SPAD (53.81), followed by ABA @ 1.5 ppm (T_4) at 49.64. Intermediate values were noted in the kinetin treatment (T_2, 36.80) and the conventional Kashmir control (T_5, 30.75), which did not differ significantly from T_1. A Critical Difference (CD) of 4.57 at $p \leq 0.05$ indicates that treatments differing by more than this threshold are statistically distinct.

Table 4.3 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on leaf chlorophyll content (SPAD) of *Crocus sativus*.

Method & Location	Treatments	Leaf chlorophyll content (SPAD)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	30.61
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	36.80
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	53.81
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	49.64
Conventional (Kashmir)	T_5 (No PGRs)	30.75
Conventional (Kishtwar)	T_6 (No PGRs)	56.24
CD ($p \leq 0.05$)		4.57

4.1.4 Fresh weight of leaves

As shown in table 4.4, **f**resh weight of leaves varied only slightly across treatments, ranging from a minimum of 15.03 g $corm^{-1}$ in plants treated with kinetin @ 1 ppm (T_2) to a maximum of 16.09 g $corm^{-1}$ in the conventional Kishtwar control (T6). With a critical difference (CD) of 0.21 g at $p \leq 0.05$, the highest value at T_6 was significantly greater than the lowest at T_2 (difference = 1.06 g > CD) and also exceeded those at GA_3 @ 50 ppm (15.22 g), ABA @ 1.5 ppm (15.41 g) and IAA @ 1 ppm (15.58 g). In contrast, the pairwise

differences among IAA (T_1), ABA (T_4) and the conventional Kashmir control (T_5, 15.63 g) were all below the CD and thus non-significant, as were those between T_2 and GA_3 (T3, difference = 0.19 g), and between GA_3 and ABA (difference = 0.19 g)

4.1.5 Dry weight of leaves

As depicted in table 4.4, it showed a much wider spread, from a minimum of 2.16 g corm^{-1} under ABA @ 1.5 ppm (T_4) to a maximum of 7.91 g corm^{-1} with IAA @ 1 ppm (T_1). The CD for dry weight was 0.80 g. The peak at T_1 was significantly higher than the dry weights at GA_3 (6.45 g), the Kashmir control (5.13 g) and the Kishtwar control (6.31 g), but did not differ significantly from kinetin (T_2, 7.53 g; difference = 0.38 g < CD). Likewise, GA_3 (T_3) and the Kishtwar control (T_6) did not differ (6.45 vs 6.31 g; difference = 0.14 g < CD), whereas the minimum at ABA (T_4) was significantly lower than all other treatments

Table 4.4 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh and dry weight of leaves (g corm^{-1}) of *Crocus sativus*.

Method & Location	Treatments	Fresh weight of leaves (g corm^{-1})	Dry wt.t of leaves (g corm^{-1})
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	15.58	7.91
Hydroponic (Jammu)	T_2 (1 ppm Kinetin) + Nutrient solution	15.03	7.53
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	15.22	6.45
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	15.41	2.16
Conventional (Kashmir)	T_5 (No PGRs)	15.63	5.13
Conventional (Kishtwar)	T_6 (No PGRs)	16.09	6.31
CD (p≤0.05)		**0.21**	**0.80**

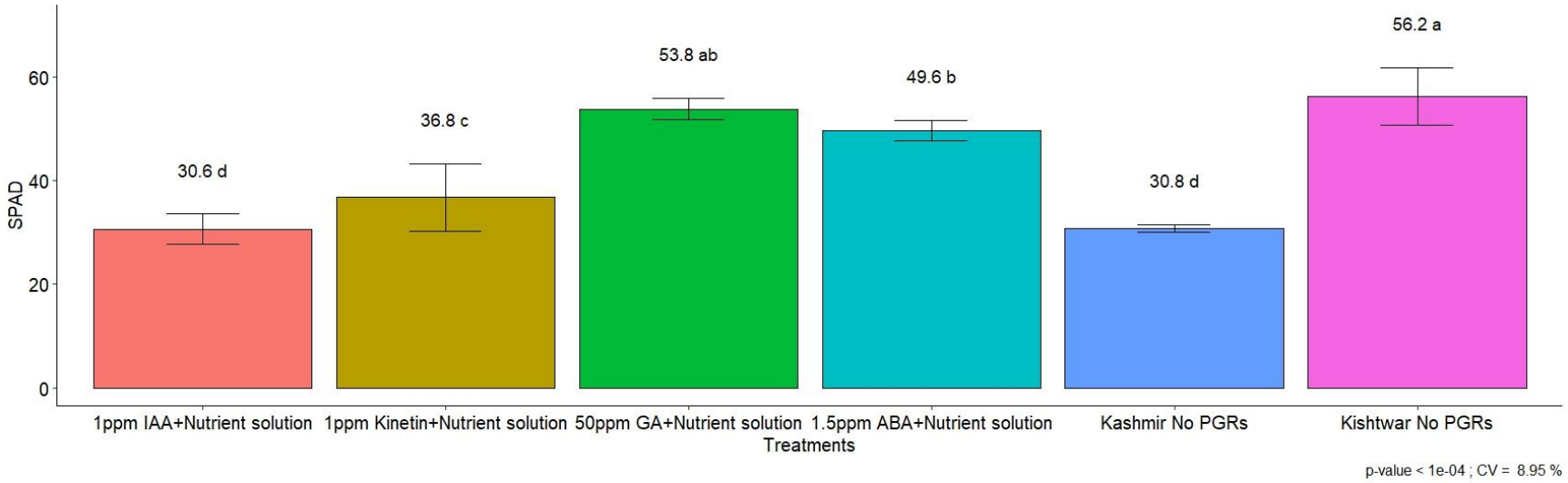

Fig. 4.3 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on leaf chlorophyll content (SPAD) of *Crocus sativus*

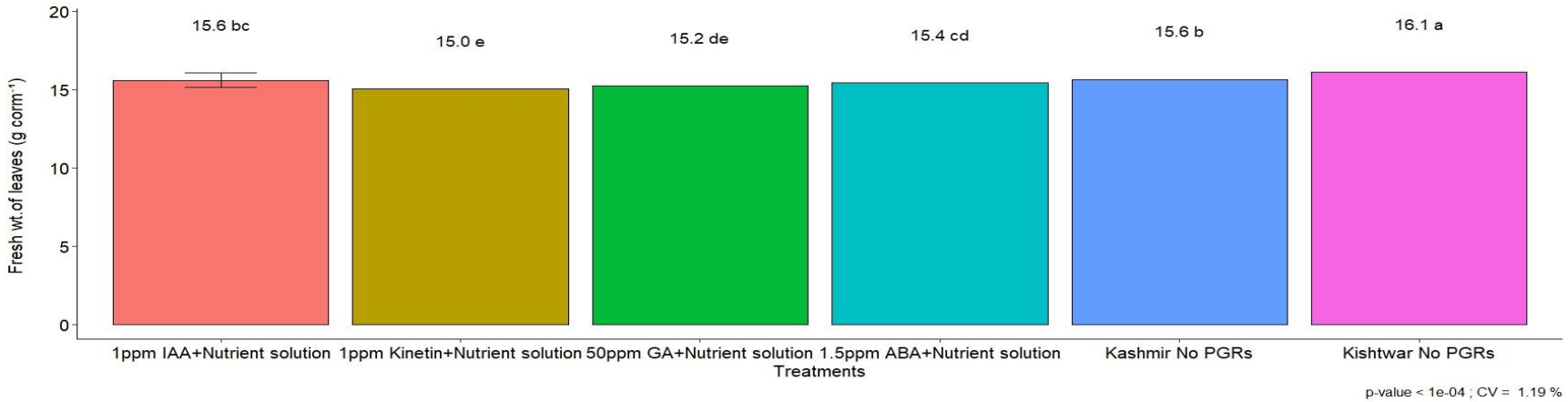

Fig. 4.4 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh weight of leaves (g corm^{-1}) of *Crocus sativus*

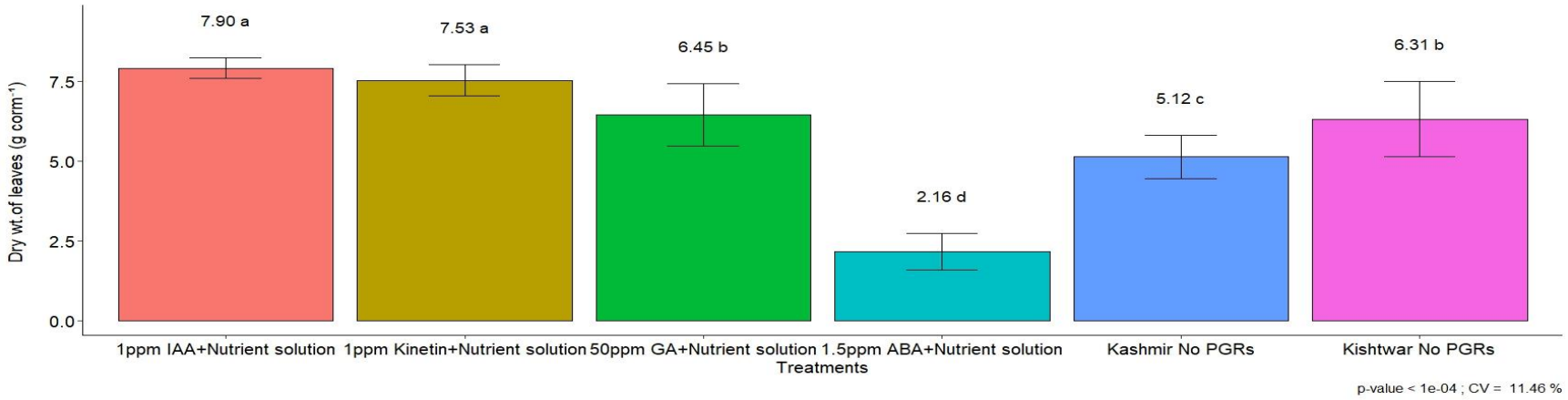

Fig. 4.5 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on dry weight of leaves (g corm^{-1}) of *Crocus sativus*

4.2 Floral attributes

4.2.1 Weight of flower

Weight of flower per corm (mg corm^{-1}) differed markedly among treatments exhibiting both a clear minimum and maximum as shown in the table 4.5. The minimum flower weight of 198.55 mg corm^{-1} was recorded in hydroponically grown plants treated with IAA @ 1 ppm (T_1), whereas the maximum of 427.49 mg corm^{-1} occurred in the conventional Kishtwar control (T_6). GA_3 @ 50 ppm (T_3) and the conventional Kashmir control (T_5) produced similarly high weights of 404.61 and 391.73 mg corm^{-1}, respectively, while kinetin @ 1 ppm (T_2) yielded an intermediate value (315.14 mg corm^{-1}) and ABA @ 1.5 ppm (T_4) gave 203.60 mg corm^{-1}. A critical difference (CD) of 127.27 mg at $p \leq 0.05$ indicated that the three highest treatments (T_3, T_5 and T_6) were each significantly greater than the two lowest (T_1 and T_4) differences exceeding the CD whereas the intermediate kinetin treatment did not differ significantly from any other group (all pairwise differences < CD). Likewise, T_1 and T_4 formed a nonsignificant pair at the bottom end, and T_3, T_5 and T_6 were mutually non-significant at the top end

Table 4.5 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on weight of flower (mg 100 corm^{-1}) of *Crocus sativus*.

Method & Location	Treatments	Weight of Flower (mg 100 corm^{-1})
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	198.55
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	315.14
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	404.61
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	203.60
Conventional (Kashmir)	T_5 (No PGRs)	391.73
Conventional (Kishtwar)	T_6 (No PGRs)	427.49
CD (p≤0.05)		**127.27**

4.2.2 Pistil length

Pistil length responded markedly to the hormonal treatments (Table 4.6, Fig. 4.6), exhibiting a nearly two-fold expansion between the shortest and longest measurements.

The minimum length (3.70 cm) occurred under ABA @ 1.5 ppm (T_4), while kinetin @ 1 ppm (T_2) drove the maximum elongation (7.91 cm). GA_3 @ 50 ppm (T_3) also promoted substantial growth (7.53 cm), and IAA @ 1 ppm (T_1) produced moderately long pistils (6.85 cm). The soil-based controls Kashmir (T_5) and Kishtwar (T_6) yielded intermediate lengths of 5.56 cm and 5.90 cm, respectively, indicating that all three growth regulators boosted pistil size above baseline.

Statistical analysis using a critical difference (CD) of 0.87 cm at $p \leq 0.05$ confirmed that T_2 and T_3 pistils were each significantly longer than those of both controls and the ABA treatment (all pairwise differences $\geq$ 1.63 cm). Although the IAA treatment also surpassed the controls (differences of 1.29 cm vs. T_5 and 0.95 cm vs. T_6), its pistil length did not differ significantly from GA_3 (difference = 0.68 cm < CD), suggesting a comparable efficacy between IAA and GA_3 in enhancing floral organ development. By contrast, neither the two soil controls nor the kinetin and GA_3 treatments differed significantly among themselves (all differences < CD), underscoring that kinetin and GA_3 share a similarly strong promotive effect. The markedly reduced length under ABA highlights its inhibitory role in pistil elongation, being significantly shorter than every other treatment (all differences > CD). These findings indicate that kinetin and GA_3 can effectively enhance reproductive organ size potentially improving pollination efficiency whereas ABA may constrain pistil development under the conditions tested.

Table 4.6 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on pistil length (cm) of *Crocus sativus*.

Method & Location	Treatments	Pistil length (cm)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	6.85
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	7.91
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	7.53
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	3.70
Conventional (Kashmir)	T_5 (No PGRs)	5.56
Conventional (Kishtwar)	T_6 (No PGRs)	5.90
CD ($p \leq 0.05$)		**0.87**

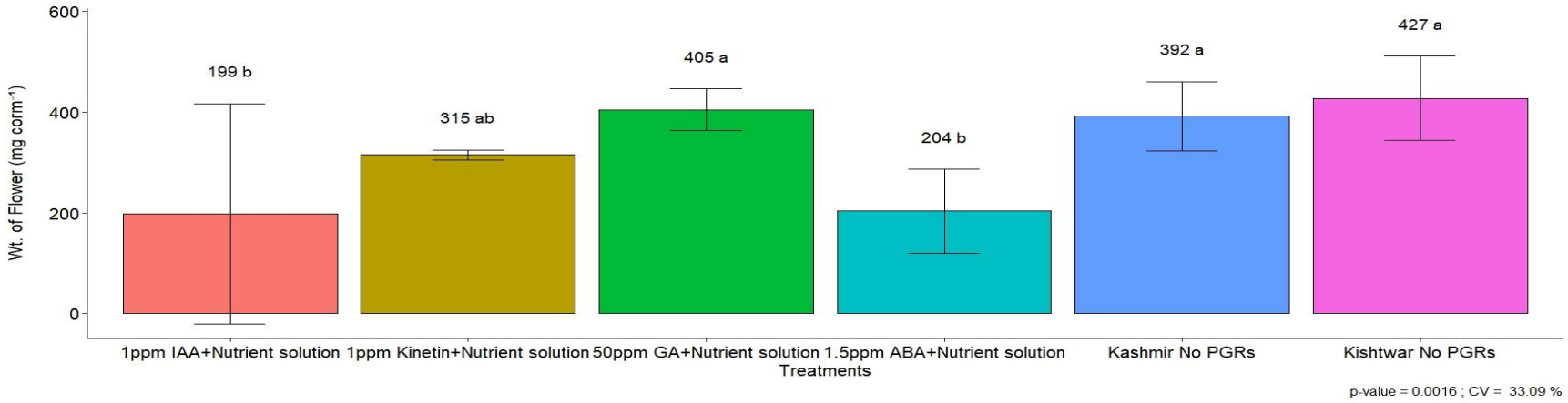

Fig. 4.6 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on weight of flower (mg corm^{-1}) of *Crocus sativus*

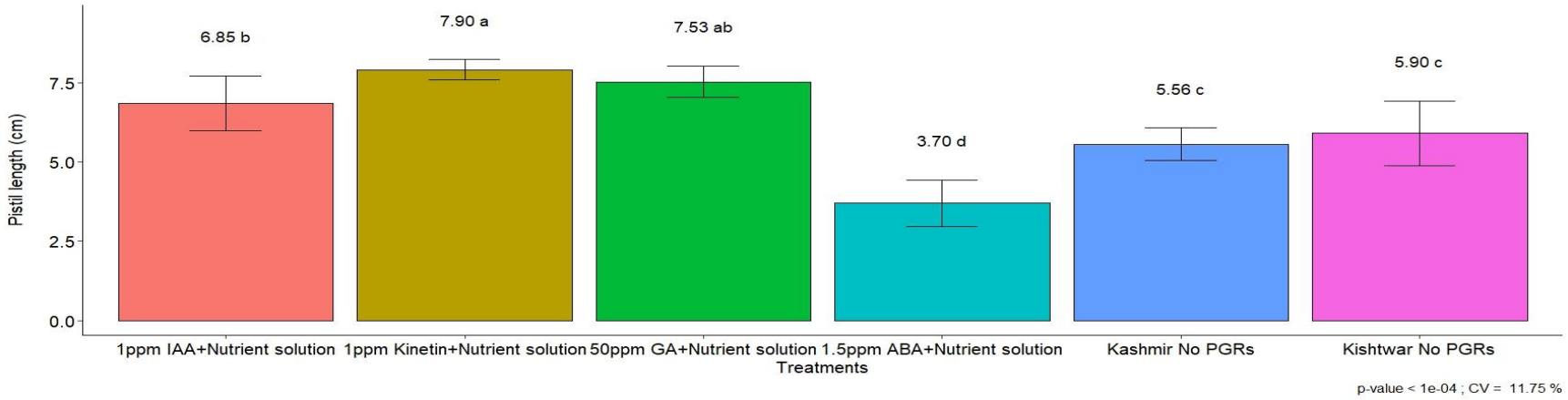

Fig. 4.7 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on pistil length (cm) of *Crocus sativus*

4.2.3 Fresh weight of pistil

Fresh pistil weight (Table 4.7, Fig. 4.7) spanned from a lowest of 2.16 g corm^{-1} under ABA @ 1.5 ppm (T_4) to a highest of 7.91 g corm^{-1} with IAA @ 1 ppm (T_1). Kinetin @ 1 ppm (T_2) yielded 7.53 g, marginally below IAA, while GA_3 @ 50 ppm (T_3) produced 6.45 g. The soil controls i.e.Kashmir (T_5) and Kishtwar (T_6)—registered 5.13 g and 6.31 g, respectively. Given the very small critical difference (CD = 0.07 g at $p \leq 0.05$), every treatment pairwise comparison exceeded this threshold, indicating that each hormone treatment elicited a distinct effect on pistil hydration.

4.2.4 Dry weight of pistil

Dry pistil weight (Table 4.7) varied from a lowest of 0.09 g corm^{-1} in T4 to a highest of 0.40 g corm^{-1} in T_1. Intermediate measurements were 0.32 g (T_2), 0.23 g (T_3), 0.12 g (T_5) and 0.30 g (T_6). However, none of these contrasts surpassed the CD of 0.80 g ($p \leq 0.05$), rendering all differences indistinguishable.

This divergence between fresh and dry mass responses implies that while plant growth regulators dramatically altered water content within pistil tissues, they did not translate into proportional changes in structural biomass deposition under the conditions tested. Such a pattern points to a primary modulation of cell hydration dynamics rather than cell wall synthesis or dry matter accumulation by the applied PGRs.

Table 4.7 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh and dry weight of pistil (g corm^{-1}) of *Crocus sativus*.

Method & Location	Treatments	Fresh weight of pistil (g corm^{-1})	Dry weight of pistil (g corm^{-1})
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	7.91	0.40
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	7.53	0.32
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	6.45	0.23
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	2.16	0.09
Conventional (Kashmir)	T_5 (No PGRs)	5.13	0.12
Conventional (Kishtwar)	T_6 (No PGRs)	6.31	0.30
CD (p≤0.05)		**0.07**	**0.80**

4.2.5 No. of flowers per corm

Flower number per corm (Table 4.8) varied markedly with treatment, ranging from a lowest count of 0.00 flowers under ABA @ 1.5 ppm (T_4) to a highest of 2.00 flowers in the conventional Kishtwar soil control (T_6). This peak was closely followed by kinetin @ 1 ppm (T_2) with 1.83 flowers and GA_3 @ 50 ppm (T_3) with 1.67 flowers per corm, while the Kashmir control (T5) produced 1.50 flowers and IAA @ 1 ppm (T_1) yielded 1.00 flower. With a critical difference (CD) of 0.59 at $p \leq 0.05$, the ABA treatment's zero flower count was significantly lower than all other treatments, indicating full inhibition of floral initiation. IAA-treated corms bore significantly fewer flowers than those under kinetin, GA_3 and the Kishtwar control (differences of 0.83, 0.67 and 1.00 flowers, respectively, all > CD), yet were statistically comparable to the Kashmir control (difference = 0.50 < CD). In contrast, differences among kinetin, GA_3, Kashmir and Kishtwar treatments fell below

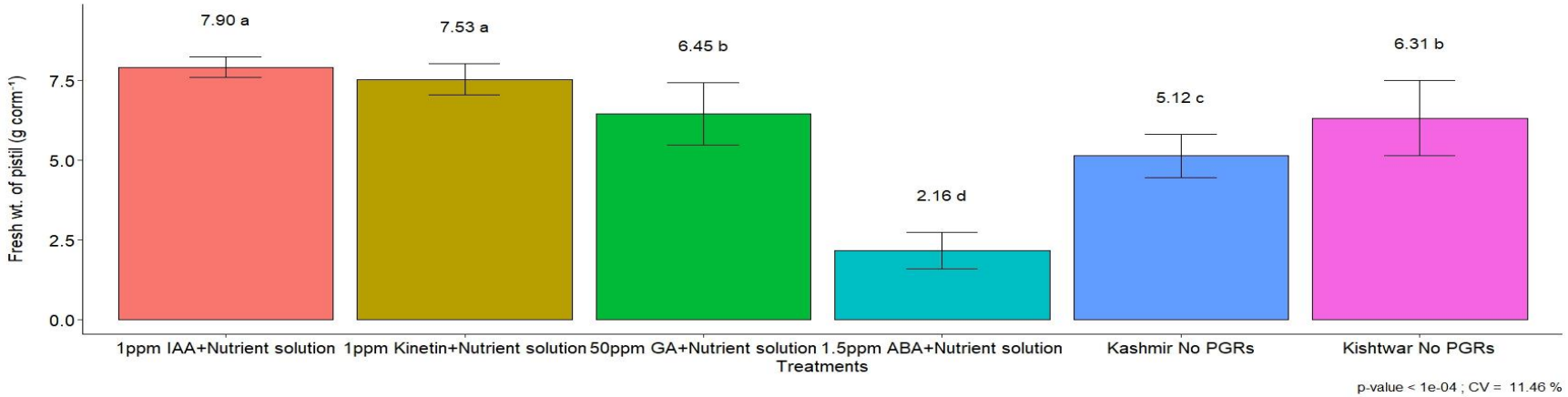

Fig. 4.8 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh weight of pistil (g corm^{-1}) of *Crocus sativus*

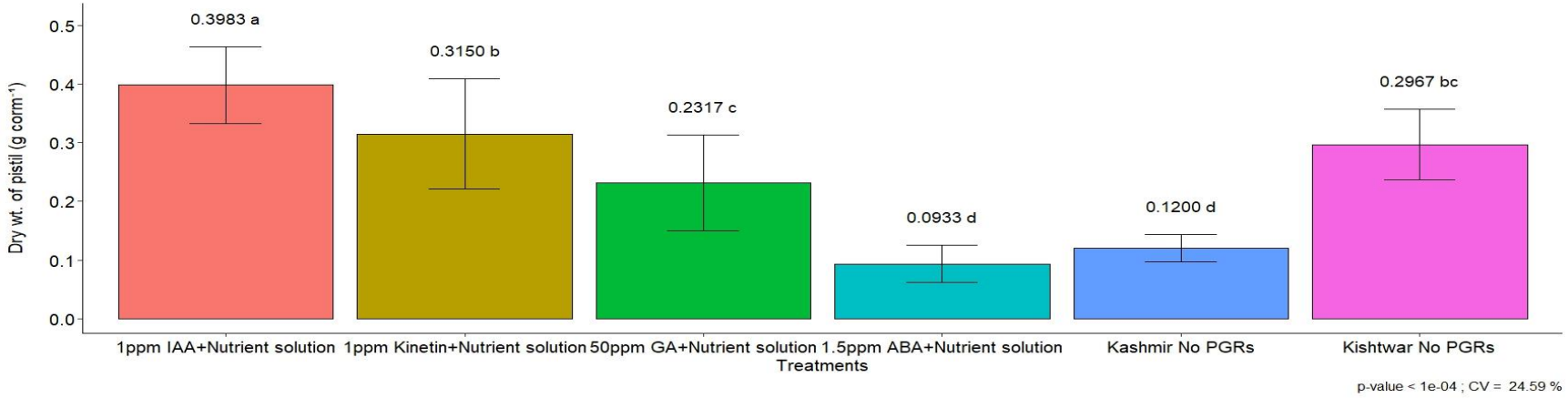

Fig. 4.9 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on dry weight of pistil (g corm^{-1}) of *Crocus sativus*

the CD threshold, revealing no significant variation in flower number among these four. Overall, kinetin and gibberellin applications matched the best soil conditions in promoting flower set, auxin had an intermediate effect, and ABA acted as a strong suppressor of flowering under the conditions tested.

Table 4.8 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of flowers per corm of *Crocus sativus*.

Method & Location	Treatments	No. of flowers per corm
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	1.00
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	1.83
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	1.67
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00
Conventional (Kashmir)	T_5 (No PGRs)	1.50
Conventional (Kishtwar)	T_6 (No PGRs)	2.00
CD ($p \leq 0.05$)		**0.59**

4.2.6 Stigma length

Stigma length (Table 4.9) displayed considerable treatment-dependent variation, ranging from a complete absence of measurable stigma under ABA @ 1.5 ppm (T_4) to a pronounced elongation of 1.71 cm following GA_3 @ 50 ppm (T_3). Kinetin @ 1 ppm (T_2) produced 1.31 cm of stigma, while the soil-based controls—Kashmir (T_5) and Kishtwar (T_6) yielded 1.22 cm and 1.38 cm, respectively; IAA @ 1 ppm (T_1) fell between these at 0.89 cm. Applying a critical difference of 0.51 cm ($p \leq 0.05$) showed that the ABA treatment's lack of stigma development was statistically distinct from all other regimes, confirming its inhibitory effect. Likewise, the GA_3-induced extension was statistically distinct when compared with the IAA-mediated length, underscoring GA_3's superior promotive action. By contrast, the stigma measurements for kinetin, Kashmir and Kishtwar treatments did not differ beyond the CD threshold when compared pairwise, indicating statistically similar effects among these intermediate treatments.

Table 4.9 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on stigma length (cm) of *Crocus sativus*.

Method & Location	Treatments	Stigma Length (cm)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	0.89
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	1.31
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	1.71
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00
Conventional (Kashmir)	T_5 (No PGRs)	1.22
Conventional (Kishtwar)	T_6 (No PGRs)	1.38
CD ($p \leq 0.05$)		**0.51**

4.2.7 Fresh weight of stigma

Table 4.10 compares stigma biomass both fresh and dry across six treatments, four hydroponic (Jammu) and two conventional soil controls (Kashmir and Kishtwar). Each value is expressed per corm, and statistical separation was assessed at $p \leq 0.05$ using critical differences (CD) of 8.61 mg for fresh weight and 0.83 mg for dry weight.

Fresh stigma weight

Fresh mass varied markedly, with the ABA treatment (T_4) showing a complete lack of fresh stigma (0.00 mg), while GA_3 application (T_3) achieved the greatest hydration at 56.35 mg per corm. Kinetin (T_2) followed closely at 53.16 mg, IAA (T_1) reached 36.29 mg, and the conventional soil controls recorded intermediate values of 44.71 mg (T_5) and 45.17 mg (T_6). Because the CD is 8.61 mg, ABA (T_4) is uniquely separated at the minimal end, producing no stigma biomass. GA_3 and kinetin did not differ statistically from each other (difference = 3.19 mg < CD), indicating similar efficacy in promoting water retention. Both of these treatments generated significantly more fresh mass than IAA (differences > 8.61 mg), whereas the IAA group did not differ significantly from the Kashmir control (difference = 8.42 mg < CD) but fell behind the Kishtwar control (difference = 8.88 mg > CD). Finally, the two soil-grown controls themselves were statistically indistinct (difference = 0.46 mg < CD).

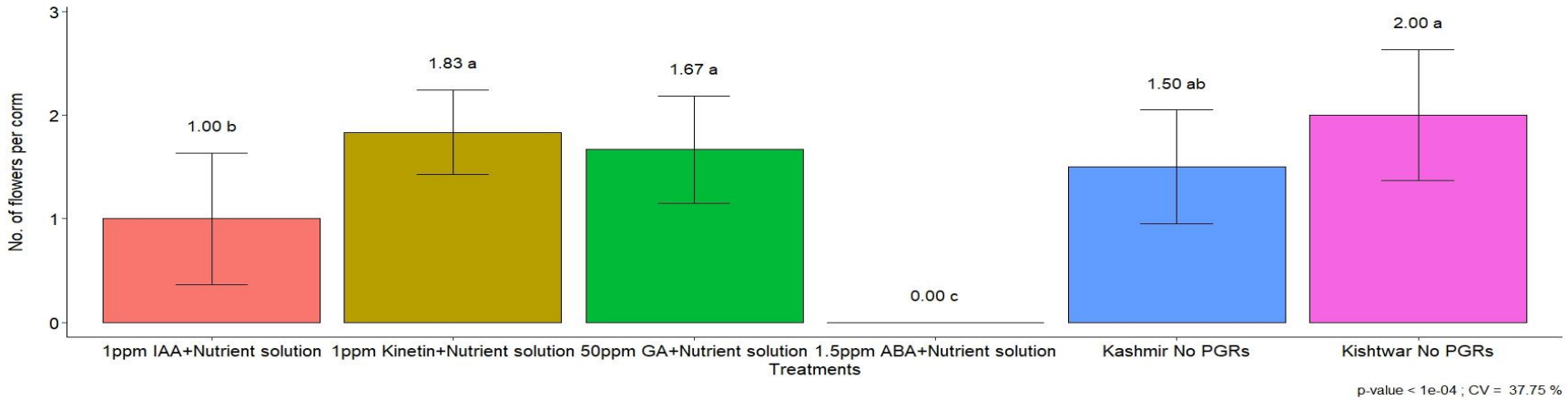

Fig. 4.10 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of flowers per corm of *Crocus sativus*

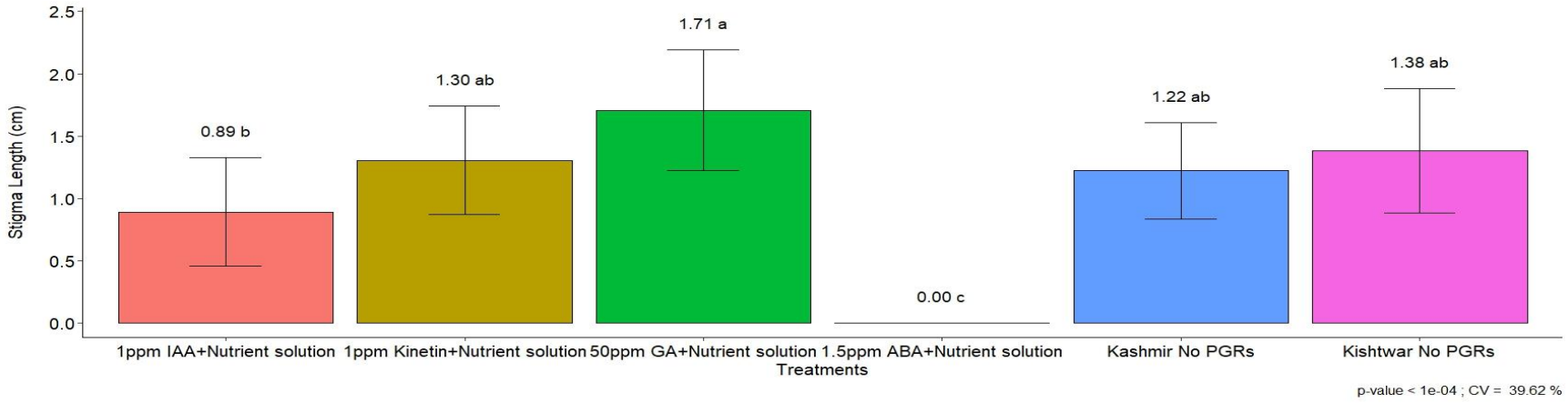

Fig. 4.11 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on stigma length (cm) of *Crocus sativus*

4.2.8 Dry weight of stigma

Dry mass spanned from no detectable tissue under ABA (T_4) to 5.42 mg per corm in the Kishtwar control (T_6). IAA (T_1) produced 3.08 mg, kinetin (T_2) 4.89 mg, GA_3 (T_3) 5.31 mg, and the Kashmir control (T_5) 5.15 mg. With a CD of 0.83 mg, ABA again stands alone at the minimal end. IAA was also significantly lower than every other treatment (differences ≥ 2.07 mg > CD), demonstrating its relatively weak effect on structural biomass deposition. In contrast, kinetin, GA_3, and both soil controls did not differ beyond the CD threshold (all pairwise differences < 0.83 mg), forming a homogeneous group in their dry stigma accumulation.

Together, these results reveal that ABA completely suppresses stigma development, IAA moderately enhances water uptake but limits dry-matter synthesis, and both kinetin and GA_3 outperform conventional soil conditions in fresh biomass while matching them in dry mass.

Table 4.10 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh and dry weight of stigma (mg per corm) of *Crocus sativus*.

Method & Location	Treatments	Fresh weight of stigma (mg per 100 corm)	Dry weight of stigma (mg per 100 corm)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	36.29	3.08
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	53.16	4.89
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	56.35	5.31
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00	0.00
Conventional (Kashmir)	T_5 (No PGRs)	44.71	5.15
Conventional (Kishtwar)	T_6 (No PGRs)	45.17	5.42
CD (p≤0.05)		**8.61**	**0.83**

4.2.9 Total dry yield of stigma

Table 4.11 presents the total dry stigma yield per 100 corms under six different treatments, with pairwise comparisons judged at $p \leq 0.05$ (CD = 1.02 g).

In corms treated with 1 ppm IAA (T_1), stigma dry yield was 2.27 g more than double the zero observed when ABA @ 1.5 ppm was applied (T_4). 1 ppm Kinetin, T_2 produced the greatest yield at 7.65 g, closely followed by gibberellin treatment (50 ppm GA_3, T_3) which gave 6.83 g. The two soil controls also clustered near the upper end, with 6.63 g in the Kashmir control (T_5) and 6.67 g in the Kishtwar control (T_6).

Because the CD is 1.02 g ($p \leq 0.05$), any pairwise difference in stigma yield exceeding this value denotes a statistically distinct outcome. The zero yield under ABA (T_4) is separated from all other treatments by margins of 2.27–7.65 g, confirming its uniquely suppressed output. IAA (T_1) at 2.27 g falls at least 4.36 g below the 6.63–7.65 g range seen in kinetin (T_2), GA_3 (T_3) and the two soil controls (T_5, T_6), marking it as significantly lower. By contrast, the yields of T_2 (7.65 g), T_3 (6.83 g), T_5 (6.63 g) and T_6 (6.67 g) differ by no more than 1.02 g, rendering these four treatments statistically indistinguishable in their total dry stigma yield

Table 4.11 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on total dry yield of stigma (g) per 100 corms of *Crocus sativus*.

Method & Location	Treatments	Total dry yield of stigma (g) per 100 corms
Hydroponic (Jammu)	T1 (1 ppm IAA) + Nutrient solution	2.27
Hydroponic (Jammu)	T2 (1 ppm Kinetin)+ Nutrient solution	7.65
Hydroponic (Jammu)	T3 (50 ppm GA_3) + Nutrient solution	6.83
Hydroponic (Jammu)	T4 (1.5 ppm ABA) + Nutrient solution	0.00
Conventional (Kashmir)	T5 (No PGRs)	6.63
Conventional (Kishtwar)	T6 (No PGRs)	6.67
CD ($p \leq 0.05$)		**1.02**

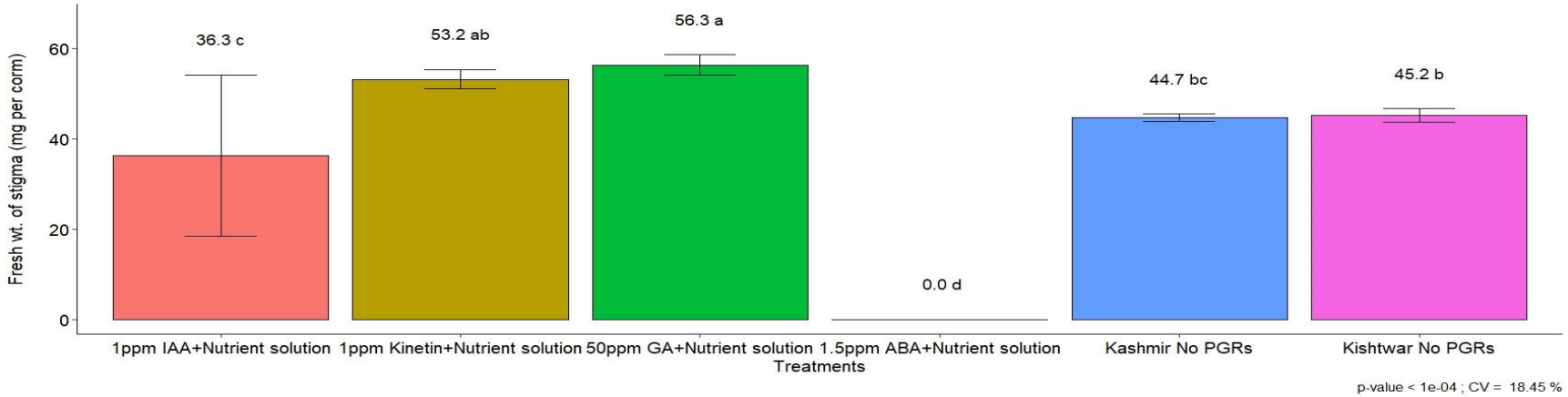

Fig. 4.12 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on fresh weight of stigma (mg per corm) of *Crocus sativus*

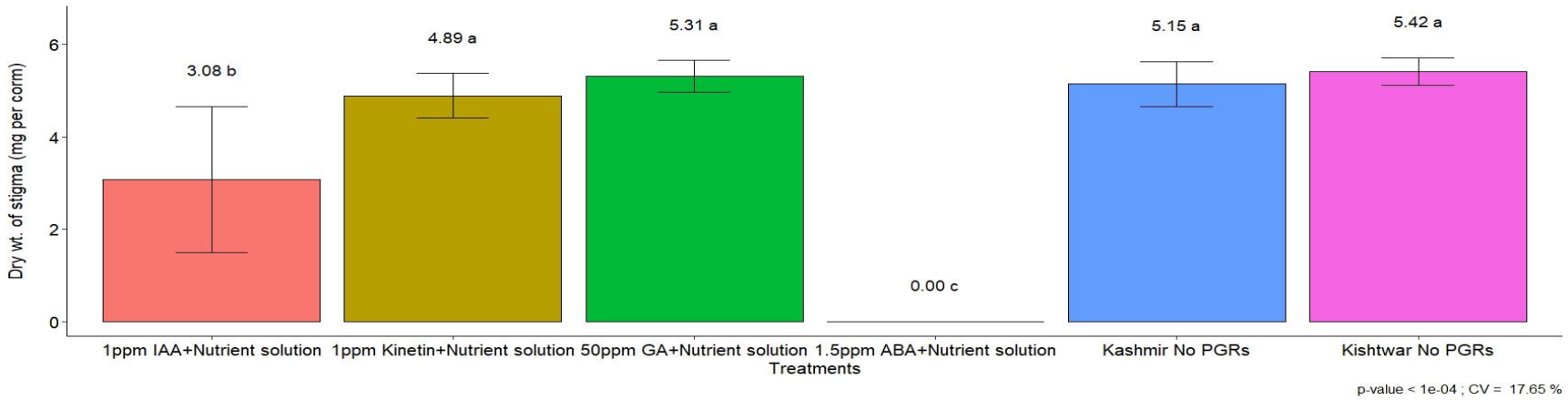

Fig. 4.13 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on dry weight of stigma (mg per corm) of *Crocus sativus*

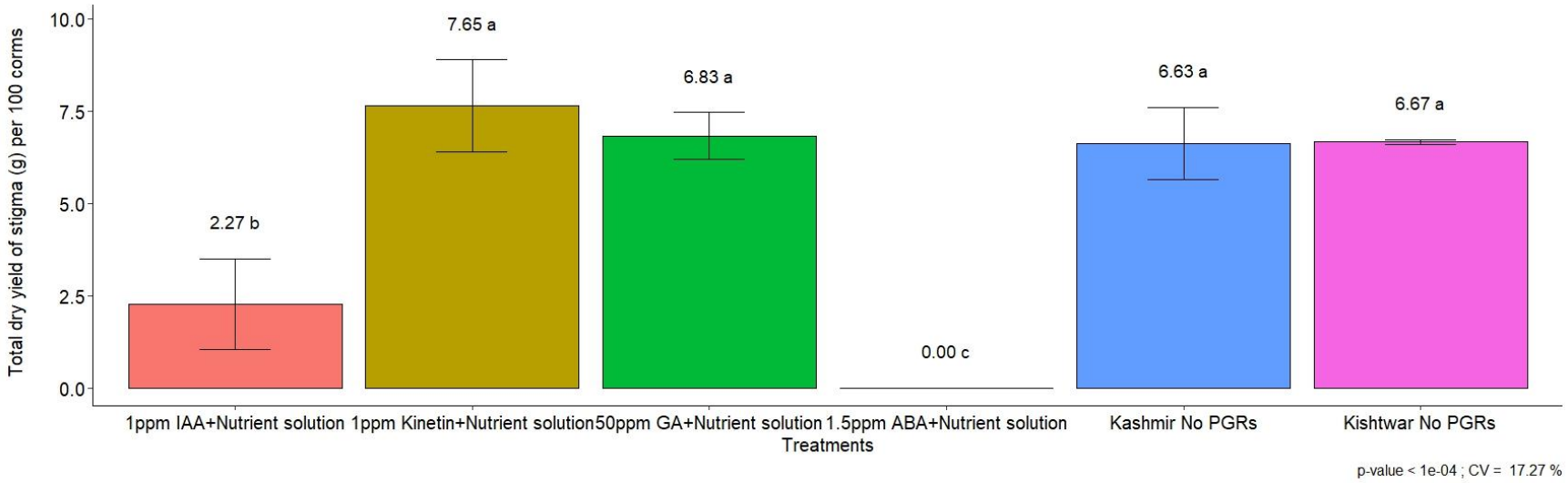

Fig. 4.14 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on total dry yield of stigma (g) per 100 corms of *Crocus sativus*

4.3 Corm attributes

4.3.1 Number of daughter corms per mother corm

Table 4.12 showed that no. of daughter corm proliferation and mean corm weight per mother corm across six treatments, with critical differences (CD) of 1.64 corms and 0.52 g at $p \leq 0.05$. In terms of number of daughter corms per mother corm, ABA @ 1.5 ppm (T_4) yielded the fewest daughter corms (2.00), indicating the weakest proliferative response, whereas IAA @ 1 ppm (T_1) generated the greatest output (8.17 corms), closely followed by kinetin @ 1 ppm (T_2) at 8.00 corms. GA_3 @ 50 ppm (T_3) produced a moderate 4.33 corms, and the soil-based controls recorded intermediate values of 6.83 corms in Kashmir (T_5) and 7.83 corms in Kishtwar (T_6). Because differences between ABA and each of the other treatments exceeded the CD of 1.64, ABA's effect is clearly distinct, and both IAA and kinetin differ sharply from GA_3 and the controls.

4.3.2 Average wt. of daughter corms

Average daughter corm weight exhibited considerable variation among treatments, spanning from a lightest mean mass of 0.58 g $corm^{-1}$ under ABA @ 1.5 ppm (T4) to a heaviest 3.03 g $corm^{-1}$ with kinetin @ 1 ppm (T_2) (Table 4.12) . IAA @ 1 ppm (T_1) yielded 2.55 g, GA_3 @ 50 ppm (T3) produced 2.07 g, and the soil-based controls i.e. Kashmir (T_5) and Kishtwar (T_6) recorded 1.55 g and 2.38 g, respectively. With the critical difference set at 0.52 g ($p \leq 0.05$), the ABA treatment's mass was distinctly below every other, confirming its strong inhibitory effect. Kinetin's output exceeded both GA_3 and the Kishtwar control by margins larger than the CD (0.96 g and 0.65 g), marking it as significantly greater than those regimes, although it did not differ appreciably from IAA (difference = 0.48 g < CD). By contrast, IAA, GA_3 and the Kishtwar control formed a homogeneous group none of their pairwise gaps surpassed the CD indicating comparable performance. The Kashmir control, at 1.55 g, remained distinctly lower than all except ABA, as every difference exceeded the CD. These distinctions define four statistical clusters of corm weight under the various PGR and soil conditions tested.

Table 4.12 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of daughter corms per mother corm and average weight of daughter corms (g) of *Crocus sativus*.

Method & Location	Treatments	No. of daughter corms per mother corm	Average wt. of daughter corms(g)
Hydroponic (Jammu)	T1 (1 ppm IAA) + Nutrient solution	8.17	2.55
Hydroponic (Jammu)	T2 (1 ppm Kinetin)+ Nutrient solution	8.00	3.03
Hydroponic (Jammu)	T3 (50 ppm GA_3) + Nutrient solution	4.33	2.07
Hydroponic (Jammu)	T4 (1.5 ppm ABA) + Nutrient solution	2.00	0.58
Conventional (Kashmir)	T5 (No PGRs)	6.83	1.55
Conventional (Kishtwar)	T6 (No PGRs)	7.83	2.38
CD (p≤0.05)		**1.64**	**0.52**

4.4 Biochemical and quality parameters

4.4.1 Crocin

Crocin accumulation at 440 nm (Table 4.13) varied markedly across treatments, with the lowest mean concentration of 0.00 % (± 0.71) under ABA @ 1.5 ppm (T_4), indicating a complete blockade of pigment biosynthesis, and the highest level of 17.40 % (± 4.17) following kinetin @ 1 ppm (T_2) . The conventional Kishtwar control (T6) closely trailed kinetin at 16.50 % (± 4.06); the 0.90 % gap between T2 and T6 falls just under the critical difference (CD = 1.82 %), grouping them as the top-tier treatments. Both of these regimes outstripped the Kashmir soil control (T_5, 14.67 % ± 3.83) by margins of 2.73 % and 1.83 % each exceeding the CD signifying a statistically notable enhancement under kinetin and native Kishtwar soils. In turn, the Kashmir control surpassed GA_3 @ 50 ppm

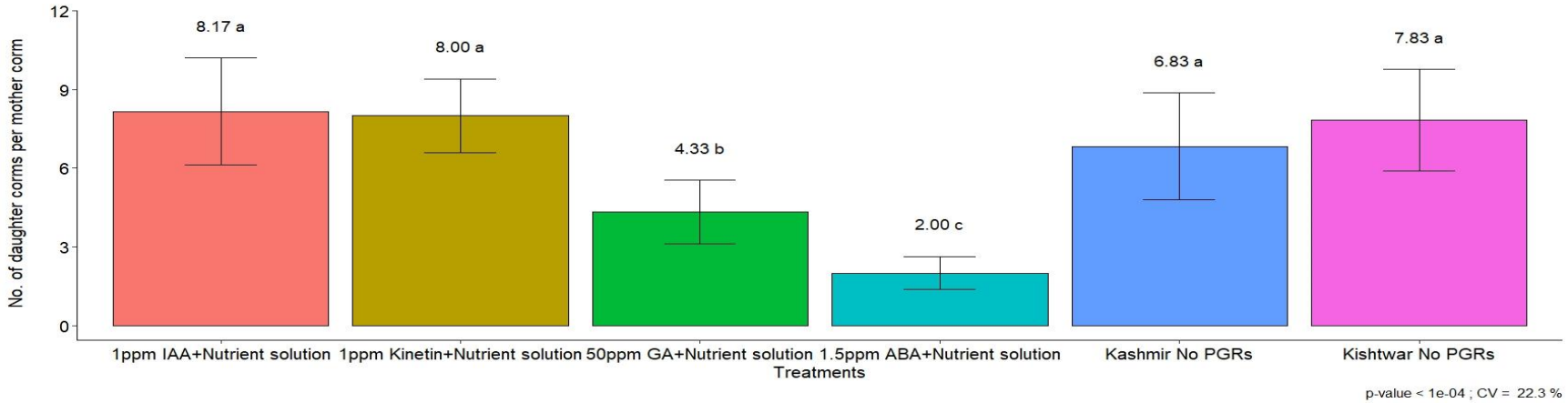

Fig. 4.15 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on no. of daughter corms per mother corm of *Crocus sativus*

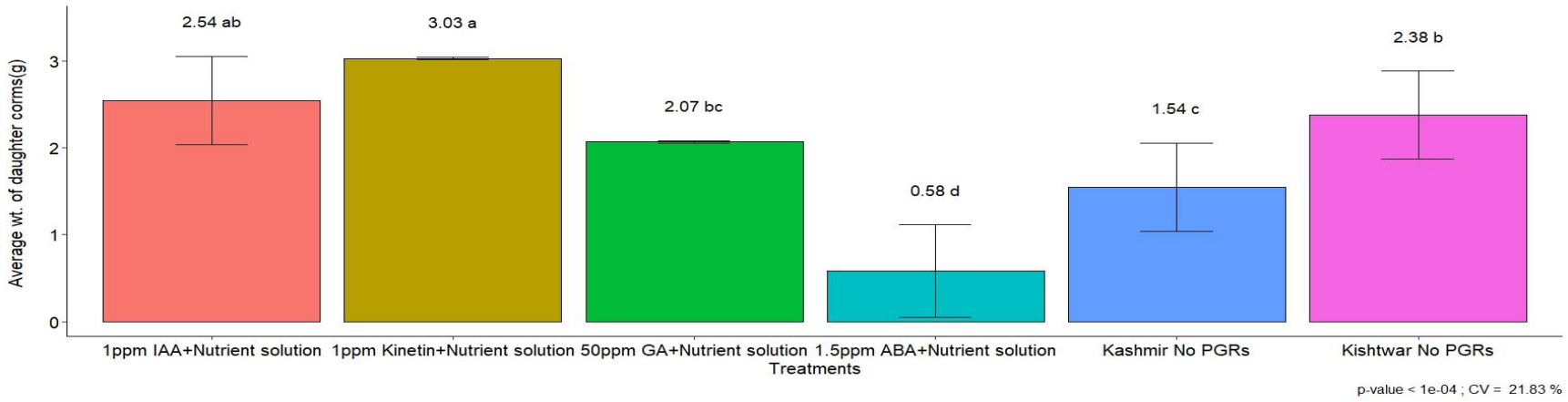

Fig. 4.16 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on average weight of daughter corms (g) of *Crocus sativus*

(T_3, 12.44 % ± 3.53) by 2.23 % and IAA @ 1 ppm (T1, 11.51 % ± 3.39) by 3.16 %, both differences exceeding the CD and thus marking T5 as distinctly more effective than the two hydroponic regulators. Meanwhile, the 0.93 % difference between GA_3 and IAA lies below the CD, indicating these two treatments yield comparable crocin levels and form a mid-tier cluster. Collectively, these results highlight that kinetin and Kishtwar soil conditions maximize crocin production, the Kashmir soil gives an intermediate boost, GA_3 and IAA perform similarly at moderate levels, and ABA fully suppresses crocin synthesis under the conditions tested.

Table 4.13 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on Crocin (%) of *Crocus sativus*.

Method & Location	Treatments	Crocin (%)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	11.51 (3.39)
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	17.40 (4.17)
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	12.44 (3.53)
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00 (0.71)
Conventional (Kashmir)	T_5 (No PGRs)	14.67 (3.83)
Conventional (Kishtwar)	T_6 (No PGRs)	16.50 (4.06)
CD (p≤0.05)		**1.82**

Note: *Figures in the parenthesis are transformed values*

4.4.2 Crocetin

Among the six regimes, the conventional Kashmir soil control (T_5) produced the most pronounced crocetin accumulation at 14.30 % (3.78), immediately followed by the

Kishtwar soil control (T_6) at 14.24 % (3.77) and the kinetin hydroponic treatment (T_2) at 13.76 % (3.71) . The differences among these top three (0.06 – 0.54 %) fall below the critical difference of 1.42 %, indicating they perform on par in driving crocetin biosynthesis. Next in effectiveness was GA_3 supplementation (T_3), which delivered 12.30 % (3.51) — a reduction of 1.46 % and 1.94 % relative to T_2 and T6, respectively, exceeding the 1.42 % threshold and thus marking GA_3's outcome as distinctly lower than the leading cluster. IAA application (T_1) yielded a more modest 9.67 % (3.19), placing it clearly beneath the GA_3 treatment (gap of 2.63 % > CD) and reflecting only moderate enhancement of pigment content. ABA treatment (T4) failed to induce any measurable crocetin (0.00 % (0.71)), confirming its strong suppressive effect in this system.

Table 4.14 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on Crocetin (%) of *Crocus sativus*.

Method & Location	Treatments	Crocetin (%)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	9.67 (3.19)
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	13.76 (3.71)
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	12.30 (3.51)
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00 (0.71)
Conventional (Kashmir)	T_5 (No PGRs)	14.30 (3.78)
Conventional (Kishtwar)	T_6 (No PGRs)	14.24 (3.77)
CD (p≤0.05)		**1.42**

Note: *Figures in the parenthesis are transformed values*

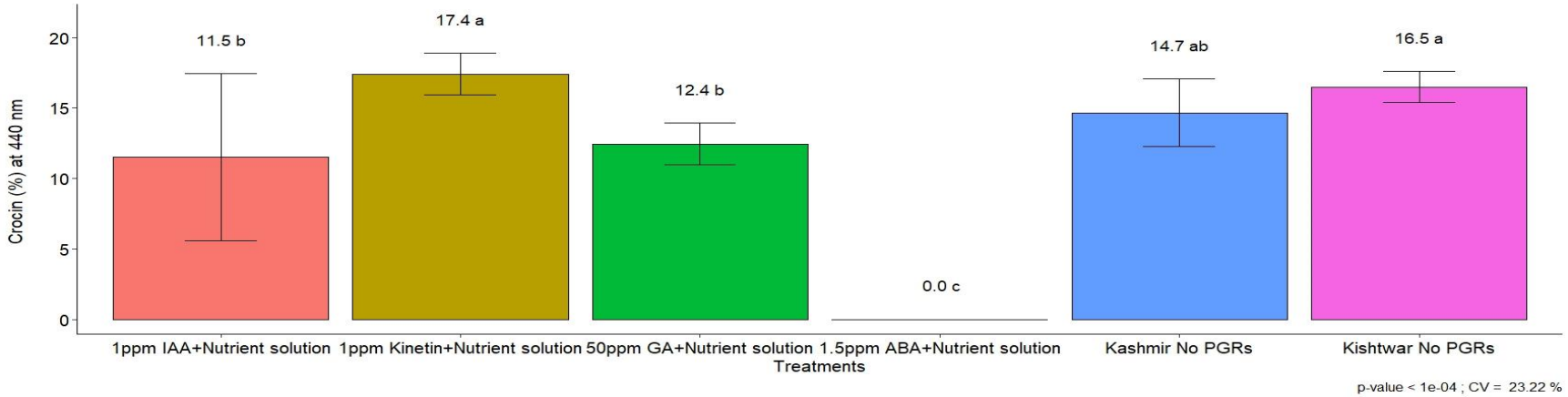

Fig. 4.17 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on Crocin (%) of *Crocus sativus*

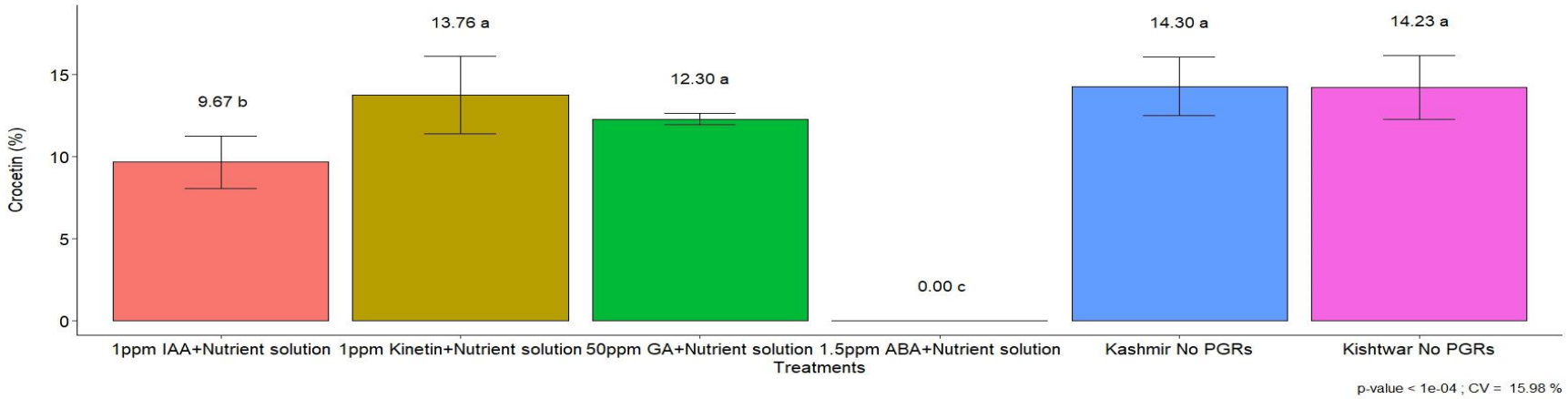

Fig. 4.18 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on Crocetin (%) of *Crocus sativus*

4.4.3 Picrocroin

Picrocrocin accumulation (Table 4.15) clustered into two statistically similar pairs and a set of distinctly different treatments. The first pair—kinetin @ 1 ppm (T_2, 6.17 %) and GA_3 @ 50 ppm (T_3, 6.03 %)—showed a difference of only 0.14 %, well below the critical difference (CD = 1.63 %), indicating a non-significant variation. Likewise, IAA @ 1 ppm (T_1, 1.18 %) versus ABA @ 1.5 ppm (T_4, 0.00 %) differed by 1.18 %, also under the CD and thus non-significant. All other pairwise comparisons including each of these four treatments against the Kashmir (T_5, 4.31 %) and Kishtwar (T_6, 5.85 %) soil controls exceeded the 1.63 % threshold, signifying significant differences in picrocrocin content among those treatments.

Table 4.15 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on picrocroin (%) of *Crocus sativus*.

Method & Location	Treatments	Picrocroin (%)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	1.18 (1.30)
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	6.17 (2.58)
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	6.03 (2.56)
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00 (0.71)
Conventional (Kashmir)	T_5 (No PGRs)	24.95 (4.99)
Conventional (Kishtwar)	T_6 (No PGRs)	15.20 (3.90)
CD (p≤0.05)		**1.63**

Note: *Figures in the parenthesis are transformed values*

4.4.4 Safranal

Table 4.16 showed safranal concentration (%) per corm (mean ± transformed value) under four hydroponic treatments in Jammu and two conventional soil controls, with a critical difference (CD) of 0.10 % at $p \leq 0.05$. In hydroponically grown corms, IAA @ 1 ppm (T_1) produced 0.02 % safranal (transformed 0.72), while both kinetin @ 1 ppm (T_2) and GA_3 @ 50 ppm (T_3) registered 0.03 % (transformed 0.73). ABA @ 1.5 ppm (T_4) completely suppressed safranal accumulation (0.00 %, transformed 0.71). Under soil-based cultivation, both the Kashmir control (T_5) and the Kishtwar control (T_6) achieved the greatest safranal levels at 0.05 % (transformed 0.74). Despite this apparent spread—from 0.00 % under ABA to 0.05 % in the soil controls—no pairwise difference exceeds the CD of 0.10 %, indicating that all six treatments yield statistically comparable safranal concentrations.

Table 4.16 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on safranal (%) of *Crocus sativus*.

Method & Location	Treatments	Safranal (%)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	0.02 (0.72)
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	0.03 (0.73)
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	0.03 (0.73)
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00 (0.71)
Conventional (Kashmir)	T_5 (No PGRs)	0.05 (0.74)
Conventional (Kishtwar)	T_6 (No PGRs)	0.05 (0.74)
CD ($p \leq 0.05$)		**0.10**

Note: *Figures in the parenthesis are transformed values*

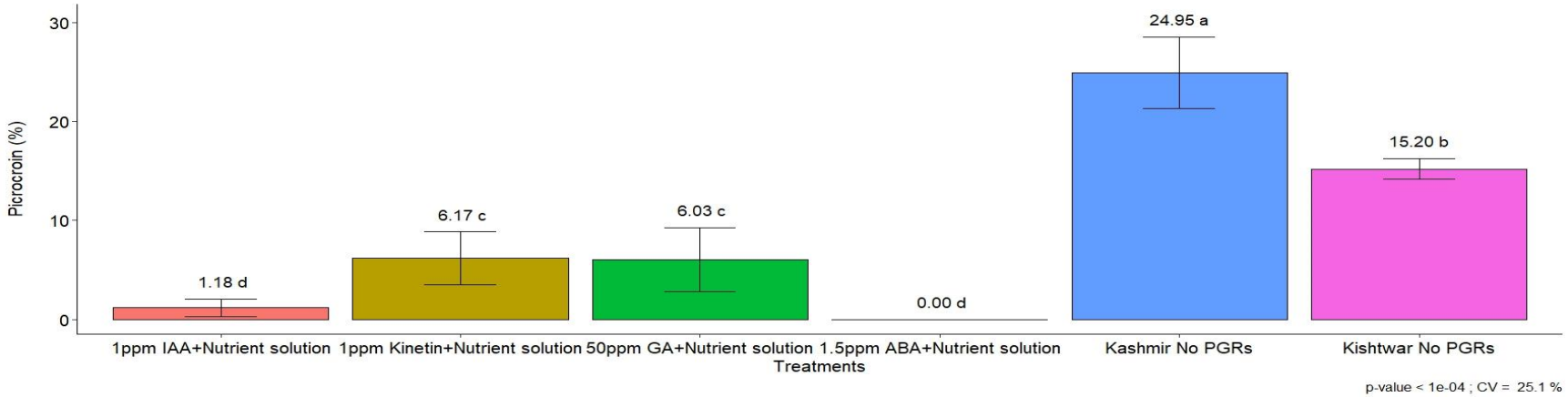

Fig. 4.19 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on picrocroin (%) of *Crocus sativus*

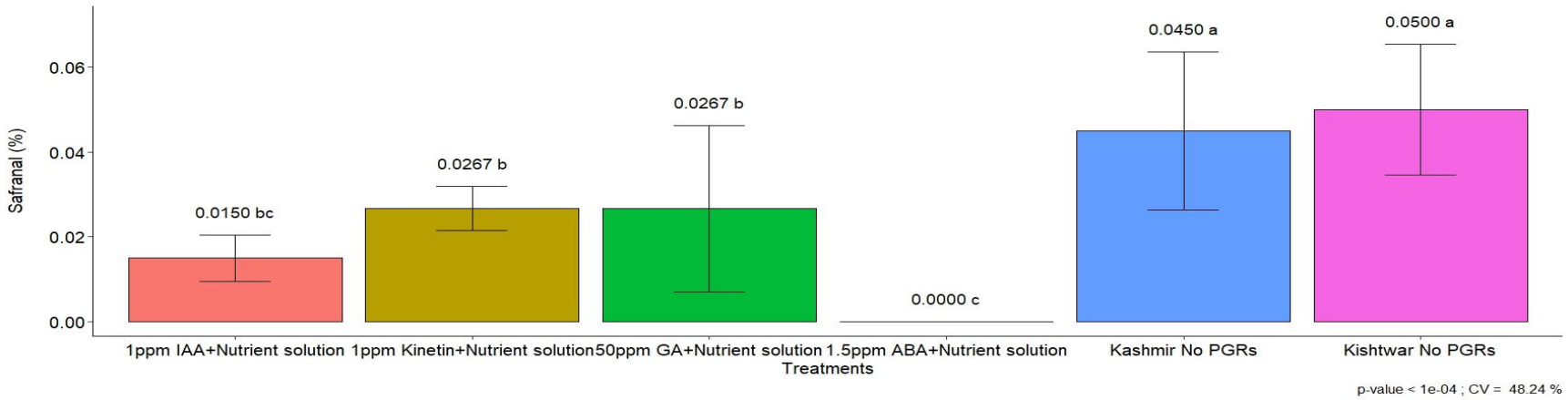

Fig. 4.20 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on safranal (%) of *Crocus sativus*

4.4.5 Flavonoid content in stigma

Flavonoid content in stigma (mg QE g^{-1} DW) varied markedly across treatments (CD = 1.60 mg). Stigmas from ABA-treated corms (T_4) contained no measurable flavonoids, whereas IAA (T_1) yielded a modest 1.12 mg. Kinetin (T_2) elevated levels to 6.31 mg, and GA_3 (T_3) further increased content to 12.12 mg. The two soil controls delivered the richest profiles, with Kashmir (T_5) at 15.63 mg and Kishtwar (T_6) at 16.63 mg. This gradient underscores a clear enhancement of flavonoid biosynthesis under cytokinin, gibberellin and native soil conditions compared to auxin or ABA treatments.

4.4.6 Flavonoid content in tepals

Flavonoid content in tepals showed the broadest spread (CD = 3.25 mg): GA_3 (T_3) recorded the lowest concentration at 10.73 mg, while ABA (T_4) drove an exceptional accumulation of 63.80 mg. IAA (T_1) and kinetin (T_2) produced intermediate values of 12.93 mg and 19.86 mg, respectively. Soil-grown flowers in Kashmir (T_5) and Kishtwar (T_6) contained 46.15 mg and 40.04 mg, respectively, placing them between the hydroponic extremes. These results highlight an unexpected stimulatory effect of ABA on tepal flavonoids under the experimental conditions.

4.4.7 Flavonoid content in leaves

Flavonoid content in leaves ranged from 5.78 mg (T_1) to 15.13 mg (T_4) with a CD of 0.80 mg. IAA-treated leaves held 5.78 mg, kinetin 11.23 mg, and GA_3 8.03 mg. Soil controls yielded 10.20 mg (T_5) and 13.59 mg (T_6). Notably, ABA again promoted the greatest accumulation in leaves, suggesting a tissue-specific induction of flavonoid synthesis by this hormone.

Table 4.17 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on flavonoid content in stigma, tepals and leaves (mg QE/g DW) of *Crocus sativus*.

Method & Location	Treatments	Flavonoid (stigma)	Flavonoid (tepals)	Flavonoid (leaves)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	1.12	12.93	5.78
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	6.31	19.86	11.23
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	12.12	10.73	8.03
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00	63.80	15.13
Conventional (Kashmir)	T_5 (No PGRs)	15.63	46.15	10.20
Conventional (Kishtwar)	T_6 (No PGRs)	16.63	40.04	13.59
CD ($p \leq 0.05$)		**1.60**	**3.25**	**0.80**

4.4.8 Phenolic content in stigma

Phenolic content in stigma tissue (Table 4.18) exhibited a wide treatment-dependent range. Application of ABA @ 1.5 ppm (T_4) effectively abolished detectable phenolics (0.00 mg GAE·g^{-1} DW), whereas kinetin @ 1 ppm (T_2) maximized accumulation at 58.22 mg GAE·g^{-1} DW. The two conventional soil controls produced intermediate profiles: 34.52 mg in the Kashmir control (T_5) and 43.91 mg in the Kishtwar control (T_6), reflecting robust baseline synthesis under native edaphic conditions. Hydroponic auxin (IAA @ 1 ppm, T_1) and gibberellin (GA_3 @ 50 ppm, T_3) treatments yielded modest phenolic levels of 18.34 mg and 19.06 mg, respectively, indicating only a minor enhancement over the ABA-suppressed baseline but substantially less than the kinetin and soil regimes. Altogether, these data demonstrate that kinetin most powerfully stimulates phenolic biosynthesis in stigma, soil-based cultivation provides a strong but lesser effect, and ABA acts as a potent inhibitor under the conditions tested.

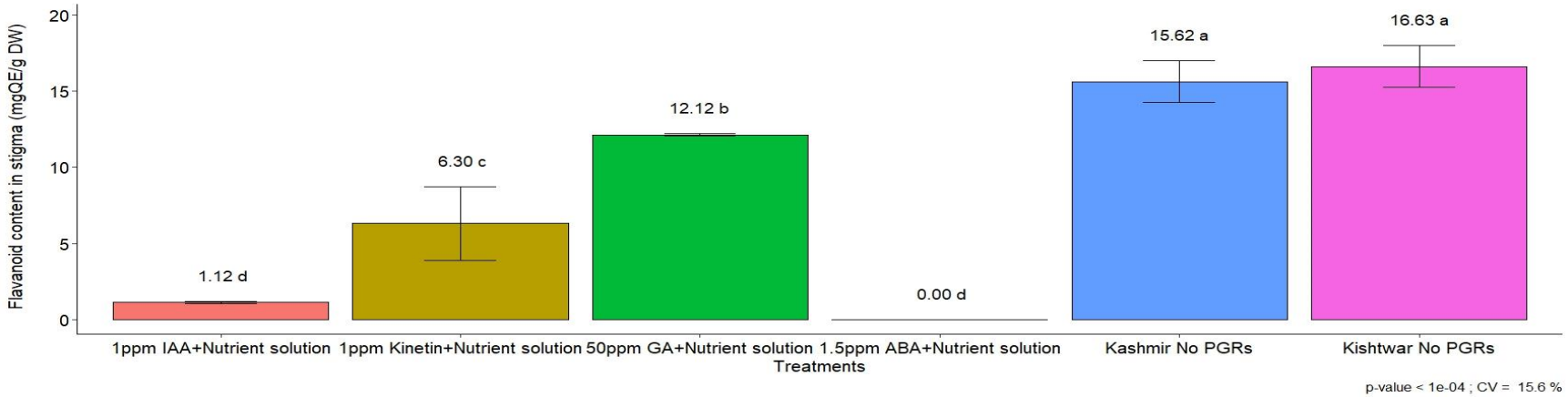

Fig. 4.21 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on flavonoid content in stigma (mg QE/g DW) of *Crocus sativus*

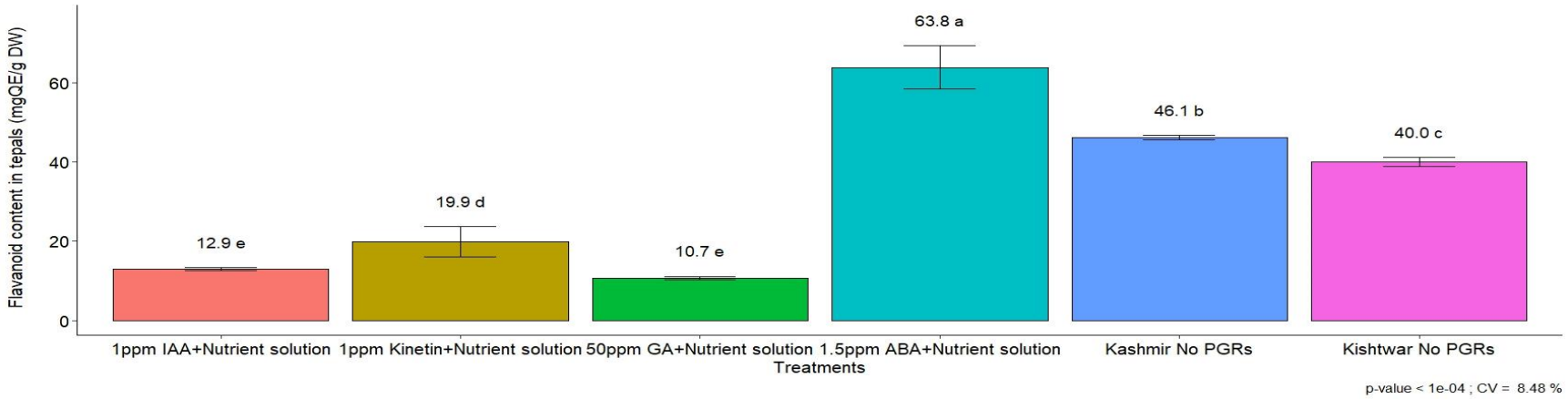

Fig. 4.22 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on flavonoid content in tepals (mg QE/g DW) of *Crocus sativus*

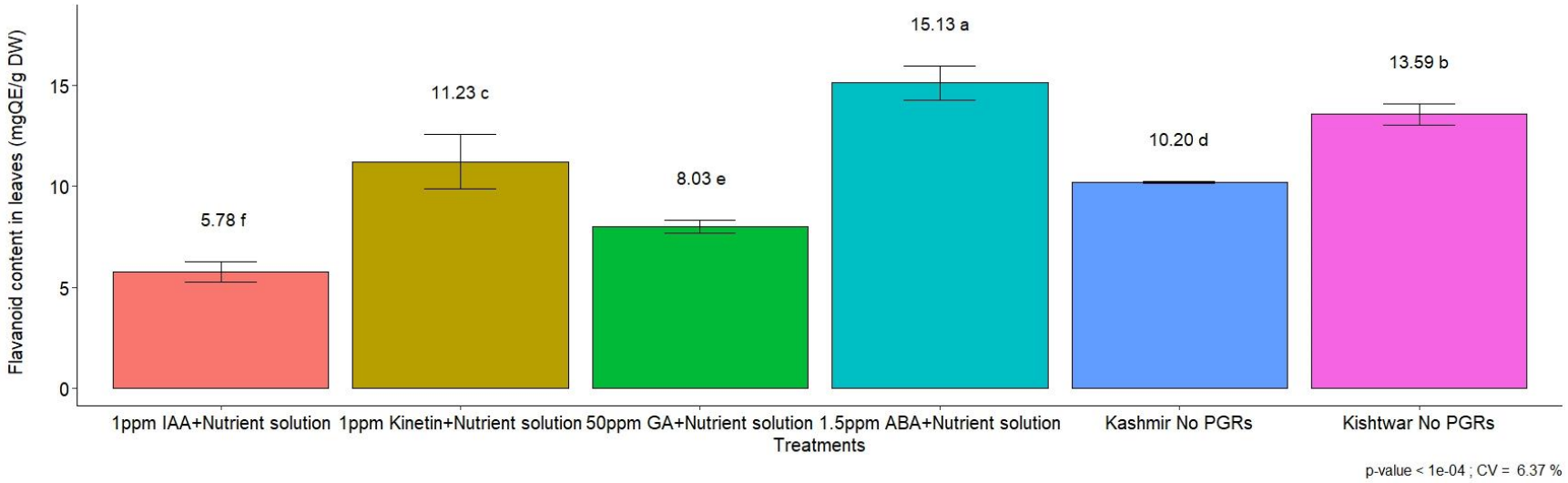

Fig. 4.23 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on flavonoid content in leaves (mg QE/g DW) of *Crocus sativus*

4.4.9 Phenolic content in tepals

The phenolic concentrations (mg GAE·g^{-1} DW) in tepals under six treatments, with a critical difference (CD) of 1.21 mg at $p \leq 0.05$.

Under hydroponic conditions in Jammu, IAA at 1 ppm (T_1) yielded 11.66 mg of phenolics, indicating a modest enhancement over baseline. Kinetin at 1 ppm (T_2) nearly doubled that amount, reaching 26.72 mg, and thereby emerging as the most effective PGR for tepal phenolics among the hormones tested. Gibberellic acid (GA_3) at 50 ppm (T_3) produced 13.66 mg—comparable to IAA but markedly lower than kinetin—while ABA at 1.5 ppm (T_4) supported 21.60 mg, suggesting a surprisingly strong promotive effect in this tissue despite its inhibitory role elsewhere.

The two soil-based controls fell between these extremes: the Kashmir control (T_5) registered 18.45 mg, and the Kishtwar control (T_6) achieved 23.91 mg of phenolics. Both soil treatments outperformed IAA and GA_3 but did not surpass kinetin, and their proximity to the kinetin value indicates that native soil factors can rival exogenous cytokinin application for phenolic synthesis in tepals. The CD of 1.21 mg confirms that the differences between kinetin (26.72 mg) and each of the other treatments ABA (21.60 mg), Kishtwar (23.91 mg), Kashmir (18.45 mg), GA_3 (13.66 mg) and IAA (11.66 mg) are all statistically meaningful, except for the 2.81 mg gap between kinetin and Kishtwar, which also exceeds the CD and thus marks kinetin as uniquely superior. Conversely, the 2.25 mg difference between ABA and the Kashmir control is likewise significant, while the smaller 0.69 mg gap between ABA and Kishtwar falls below the CD, indicating these two treatments yield comparable tepal phenolic levels. This profile underscores kinetin's potency, the robust support from Kishtwar soil, and a clear hierarchy of phenolic induction in tepals under the tested conditions.

4.4.10 Phenolic content in leaves

Leaf phenolic content (Table 4.18) displayed clear treatment-dependent variation. The least accumulation occurred with IAA @ 1 ppm (T_1), yielding 7.55 mg GAE·g^{-1} DW, whereas kinetin @ 1 ppm (T_2) drove the greatest synthesis at 19.35 mg GAE·g^{-1} DW. ABA @ 1.5 ppm (T_4) supported 14.93 mg, placing it between the extremes and indicating a

substantial upregulation relative to auxin. Among the soil controls, Kishtwar (T_6) produced 16.55 mg and Kashmir (T_5) 12.55 mg, demonstrating that native soil conditions enhance foliar phenolic levels above most hydroponic PGR treatments. GA_3 @ 50 ppm (T_3) resulted in an intermediate 10.01 mg. Overall, kinetin application most effectively stimulated phenolic deposition in leaves, with ABA and Kishtwar soil also yielding robust accumulations, while auxin treatment remained comparatively modest.

Table 4.18 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on phenolic content in stigma, tepals and leaves GAE/g DW of *Crocus sativus*.

Method & Location	Treatments	Phenolic content (stigma)	Phenolic content (tepals)	Phenolic content (leaves)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	18.34	11.66	7.55
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	58.22	26.72	19.35
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	19.06	13.66	10.01
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00	21.60	14.93
Conventional (Kashmir)	T_5 (No PGRs)	34.52	18.45	12.55
Conventional (Kishtwar)	T_6 (No PGRs)	43.91	23.91	16.55
CD ($p \leq 0.05$)		**5.80**	**1.21**	**0.95**

4.4.11 Total proline content in stigma

Stigma proline content (Table 4.19) exhibited a clear, stepwise increase across treatments, from complete absence under ABA @ 1.5 ppm (T_4) to progressive elevation under PGRs and soil controls. In IAA-treated corms (T_1), proline measured 3.26 µmol g^{-1} DW; kinetin (T_2) raised this to 4.31 µmol g^{-1}; and GA_3 (T_3) further increased it to 5.15

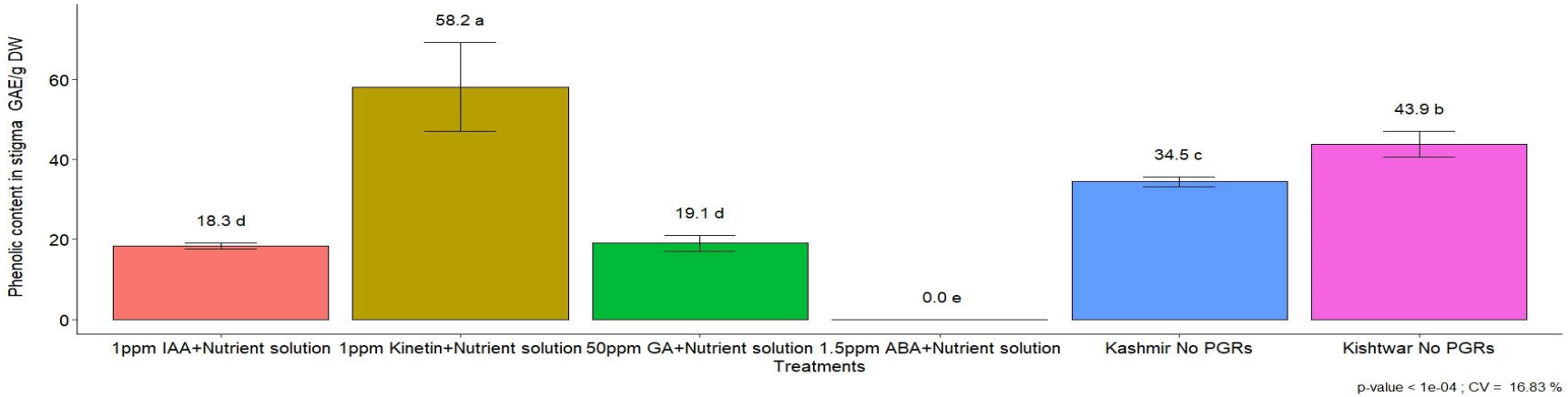

Fig. 4.24 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on phenolic content in stigma (GAE/g DW) of *Crocus sativus*

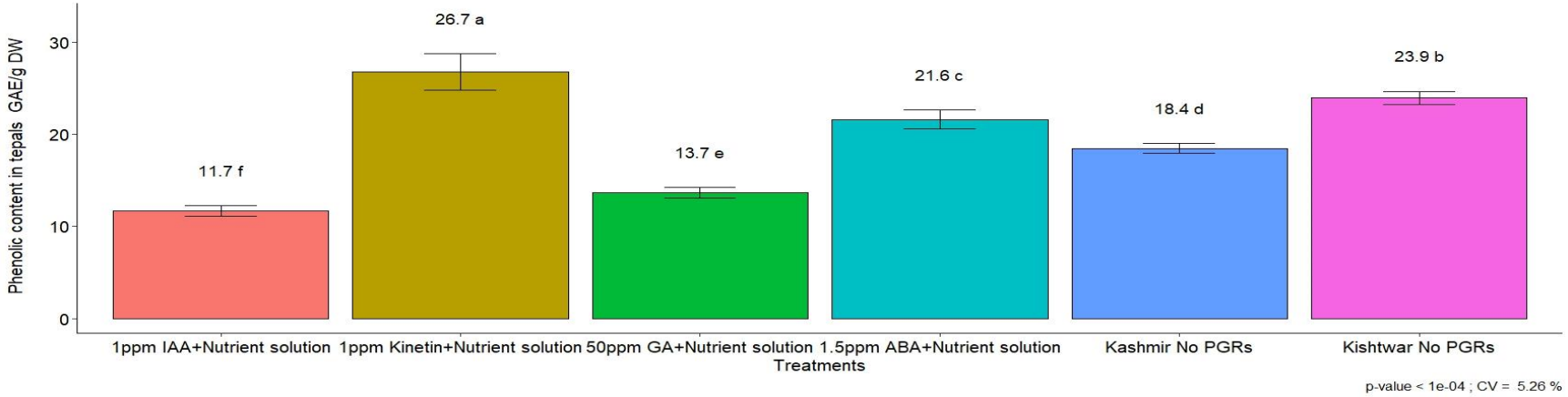

Fig. 4.25 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on phenolic content in tepals (GAE/g DW) of *Crocus sativus*

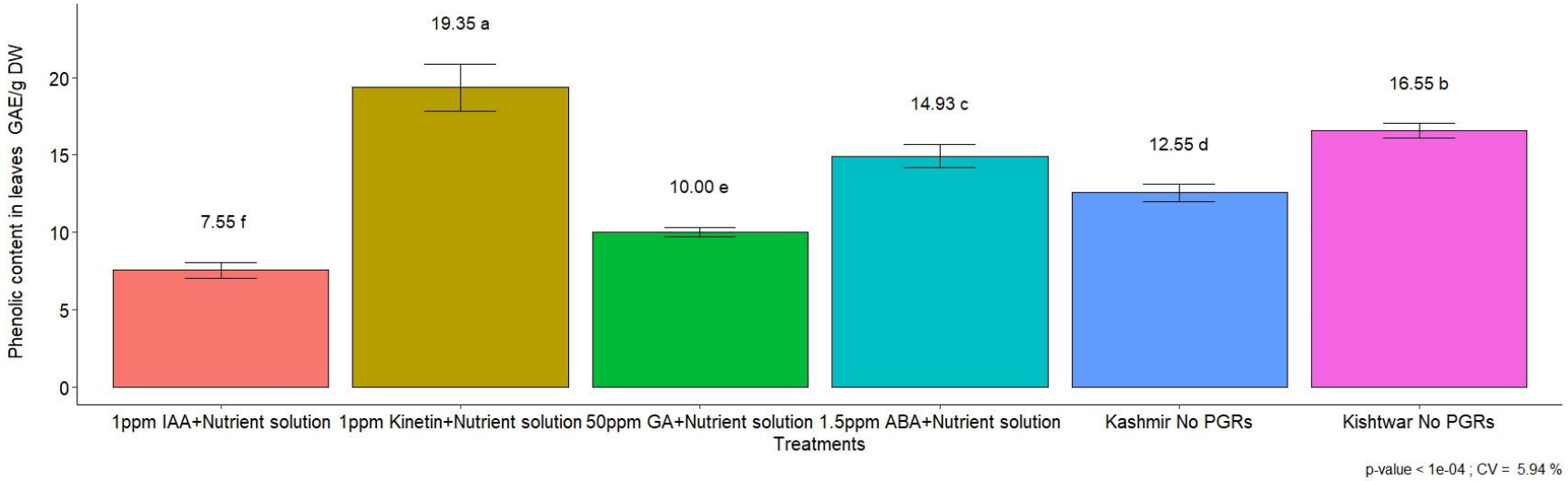

Fig. 4.26 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on phenolic content in leaves (GAE/g DW) of *Crocus sativus*

µmol g^{-1}. Under soil cultivation, the Kashmir control (T_5) achieved 6.45 µmol g^{-1}, while the Kishtwar control (T_6) reached the highest level at 7.04 µmol g^{-1}. With a critical difference of 0.08 µmol g^{-1} ($p \leq 0.05$), each adjacent treatment surpasses the threshold, confirming that every incremental rise in proline—from hormone applications through native soils—represents a statistically meaningful enhancement of osmoprotectant accumulation in stigma tissues .

4.4.12 Proline content in tepals

Tepal proline content (Table 4.19) exhibited a lowest value of 10.65 µmol g^{-1} DW under IAA @ 1 ppm (T_1) and climbed to a highest of 27.20 µmol g^{-1} DW in the Kishtwar soil control (T6). Intermediate concentrations were recorded under kinetin @ 1 ppm (T_2, 14.30 µmol g^{-1}), GA_3 @ 50 ppm (T3, 17.65 µmol g^{-1}), the Kashmir control (T_5, 21.38 µmol g^{-1}), and ABA @ 1.5 ppm (T_4, 26.21 µmol g^{-1}), illustrating a clear gradation from minimal osmoprotectant accumulation with auxin to maximal response under native Kishtwar soils.

4.4.13 Total proline content in leaves

Leaf proline accumulation in leaf tissue (Table 4.19) increased progressively across the treatments, beginning with the minimal concentration of 18.46 µmol·g^{-1} DW in IAA-treated corms (T_1) and rising to 23.36 µmol·g^{-1} under kinetin (T_2). GA_3 supplementation (T_3) further elevated proline to 29.49 µmol·g^{-1}, while ABA (T_4) drove a more pronounced accumulation of 46.26 µmol·g^{-1}. Under conventional soil cultivation, proline levels reached 38.36 µmol·g^{-1} in the Kashmir control (T_5) and peaked at 49.15 µmol·g^{-1} in the Kishtwar control (T_6), marking the maximal concentration recorded. This gradient from the hydroponic auxin baseline through cytokinin and gibberellin treatments to the highest values under ABA and native soils highlights a clear enhancement of osmoprotectant synthesis in leaf tissue across the experimental regimes

Table 4.19 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on proline content in stigma, tepals and leaves (µmol/g DW) of *Crocus sativus*.

Method & Location	Treatments	Proline (stigma)	Proline (tepals)	Proline (leaves)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	3.26	10.65	18.46
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	4.31	14.30	23.36
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	5.15	17.65	29.49
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00	26.21	46.26
Conventional (Kashmir)	T_5 (No PGRs)	6.45	21.38	38.36
Conventional (Kishtwar)	T_6 (No PGRs)	7.04	27.20	49.15
CD ($p \leq 0.05$)		**0.08**	**0.14**	**0.12**

4.4.14 DPPH content in stigma

Stigma DPPH IC_{50} (Table 4.20) displayed a pronounced treatment-dependent shift, ranging from a complete absence of measurable IC_{50} under ABA @ 1.5 ppm (T_4) to the highest value of 0.68 mg/mL with kinetin @ 1 ppm (T_2). IAA @ 1 ppm (T_1) recorded 0.45 mg/mL, GA_3 @ 50 ppm (T_3) gave 0.57 mg/mL, and the soil controls Kashmir (T_5) and Kishtwar (T_6) yielded 0.46 mg/mL and 0.54 mg/mL, respectively. Applying a critical difference of 0.10 mg/mL ($p \leq 0.05$) revealed that the ABA treatment's zero IC_{50} was statistically distinct from all other regimes, confirming its strongest radical-scavenging activity. The kinetin-induced IC_{50} was also statistically distinct when compared to every other treatment, underscoring its comparatively weak antioxidant inhibition. In contrast, the IC_{50} values for GA_3 and the Kishtwar control did not differ beyond the CD threshold (difference = 0.03 mg/mL < CD), indicating statistically similar effects, and likewise IAA and the Kashmir control were comparable (difference = 0.01 mg/mL < CD).

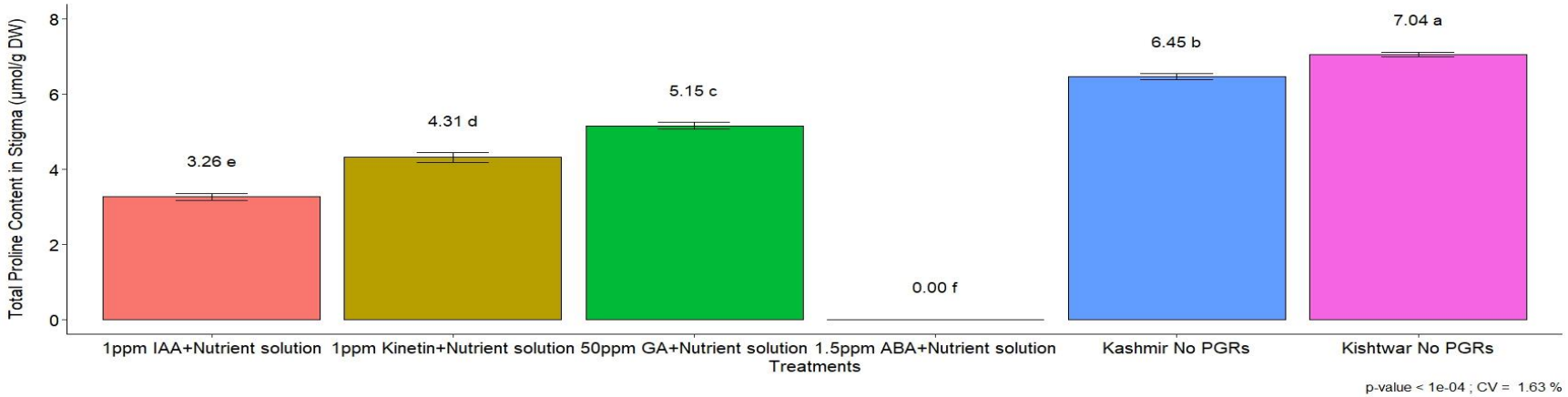

Fig. 4.27 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on proline content in stigma (µmol/g DW) of *Crocus sativus*

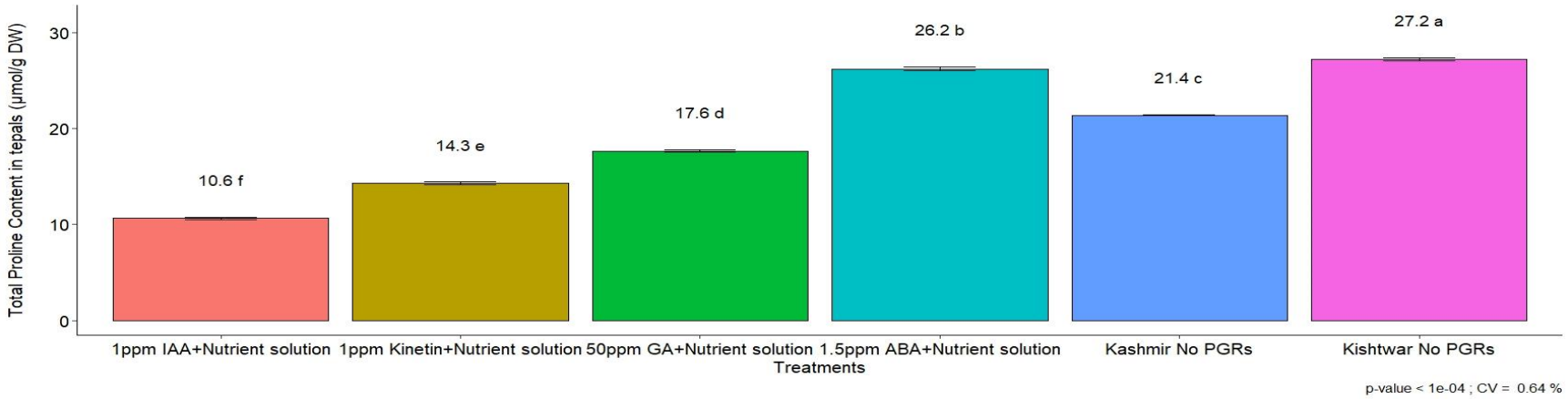

Fig. 4.28 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on proline content in tepals (µmol/g DW) of *Crocus sativus*

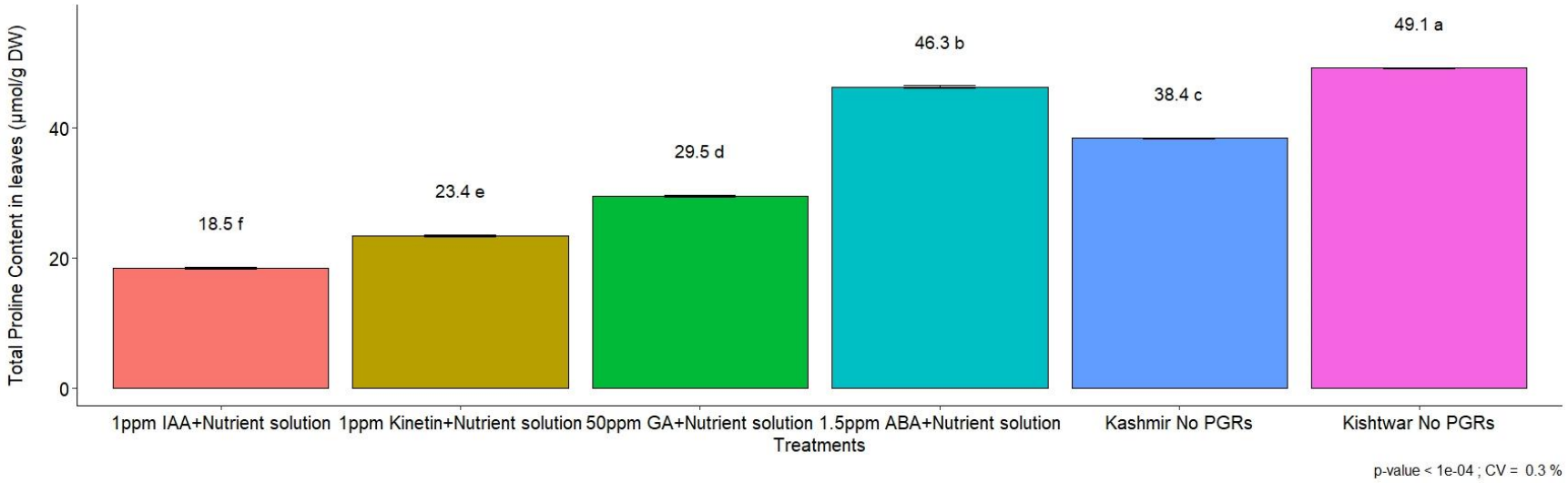

Fig. 4.29 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on proline content leaves (µmol/g DW) of *Crocus sativus*

4.4.15 DPPH content in tepal

Tepal DPPH IC_{50} (Table 4.20) varied from **a** lowest IC_{50} of 1.11 mg/mL under kinetin @ 1 ppm (T2) to a highest of 1.66 mg/mL with IAA @ 1 ppm (T_1). The other treatments were intermediate: ABA @ 1.5 ppm (T_4) at 1.27 mg/mL, the Kishtwar control (T_6) at 1.22 mg/mL, GA_3 @ 50 ppm (T_3) at 1.35 mg/mL, and the Kashmir control (T_5) at 1.46 mg/mL. A CD of 0.14 mg/mL ($p \leq 0.05$) indicated that the kinetin-treated IC_{50} was statistically distinct from those of IAA and both soil controls, highlighting its superior antioxidant efficacy in tepals. Conversely, the IC_{50} values for ABA, Kishtwar and GA_3 treatments did not exceed the CD when compared pairwise (all differences $\leq$ 0.14 mg/mL), demonstrating statistically similar scavenging capacities within this group

4.4.16 DPPH content in leaves

Leaf DPPH IC_{50} (Table 4.20) spanned from a lowest of 1.73 mg/mL under kinetin @ 1 ppm (T_2) to a highest of 2.34 mg/mL with IAA @ 1 ppm (T_1). GA_3 @ 50 ppm (T_3) and the Kishtwar control (T_6) produced 2.10 mg/mL and 1.90 mg/mL, respectively, while ABA @ 1.5 ppm (T_4) gave 1.95 mg/mL and the Kashmir control (T_5) 2.17 mg/mL. With a CD of 0.14 mg/mL ($p \leq 0.05$), the kinetin-induced IC_{50} was statistically distinct from those of GA_3, IAA and the Kashmir control, confirming its highest radical-scavenging capacity. ABA and the Kishtwar control did not differ significantly (difference = 0.05 mg/mL < CD), nor did GA_3 and the Kashmir control (difference = 0.07 mg/mL < CD), indicating comparable antioxidant effects within these pair.

Table: 4.20 Effect of application of plant growth regulators cultivated under hydroponic system v/s conventional system on DPPH content in stigma, tepals and leaves IC_{50} (mg/mL) of *Crocus sativus*.

Method & Location	Treatments	DPPH (stigma)	DPPH (tepals)	DPPH (leaves)
Hydroponic (Jammu)	T_1 (1 ppm IAA) + Nutrient solution	0.45	1.66	2.34
Hydroponic (Jammu)	T_2 (1 ppm Kinetin)+ Nutrient solution	0.68	1.11	1.73
Hydroponic (Jammu)	T_3 (50 ppm GA_3) + Nutrient solution	0.57	1.35	2.10
Hydroponic (Jammu)	T_4 (1.5 ppm ABA) + Nutrient solution	0.00	1.27	1.95
Conventional (Kashmir)	T_5 (No PGRs)	0.46	1.46	2.17
Conventional (Kishtwar)	T_6 (No PGRs)	0.54	1.22	1.90
CD ($p \leq 0.05$)		**0.10**	**0.14**	**0.14**

4.5 Principal component analysis

Table 4.5.1 PCA of treatment effects on biochemical and antioxidant traits in *Crocus sativus*

Component	Eigenvalue	Percentage of variance	Cumulative percentage of variance
Principal Component 1	5.00	71.52	71.52
Principal Component 2	1.22	17.50	89.02
Principal Component 3	0.59	8.43	97.46
Principal Component 4	0.15	2.24	99.71
Principal Component 5	0.02	0.28	100.00

In Table 4.5.1, each principal component's eigenvalue quantifies the amount of variance it captures, the percentage of variance column expresses this as a share of the total,

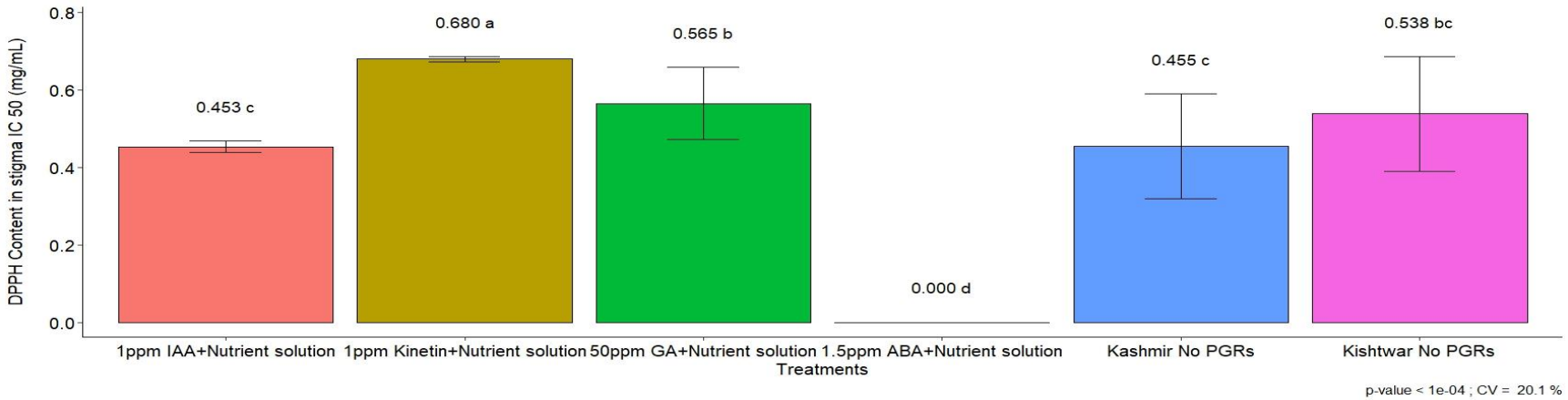

Fig. 4.30 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on DPPH content in stigma IC_{50} (mg/mL) of *Crocus sativus*

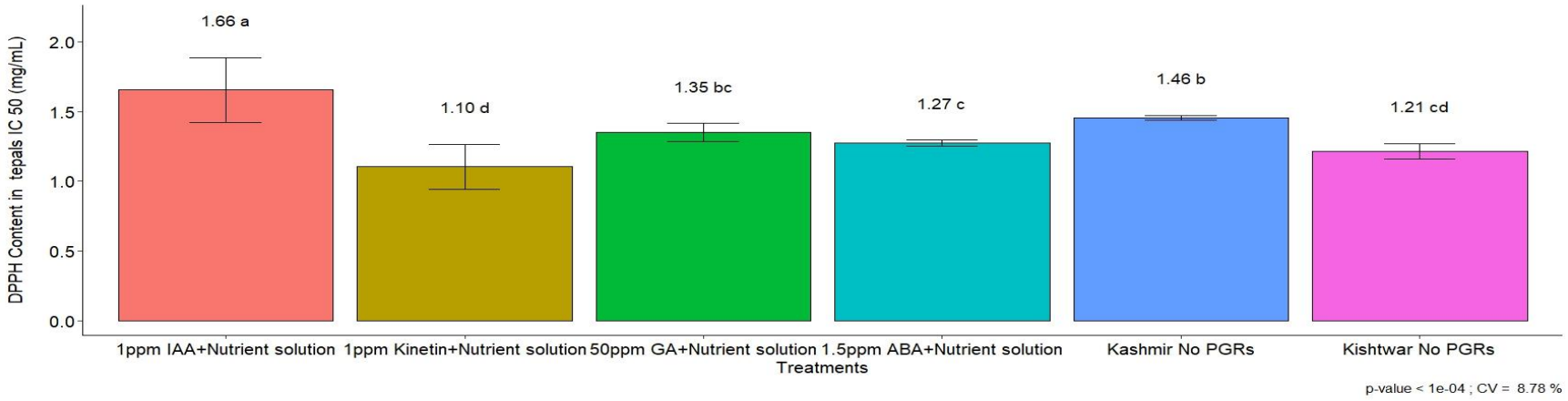

Fig. 4.31 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on DPPH content in tepals IC_{50} (mg/mL) of *Crocus sativus*

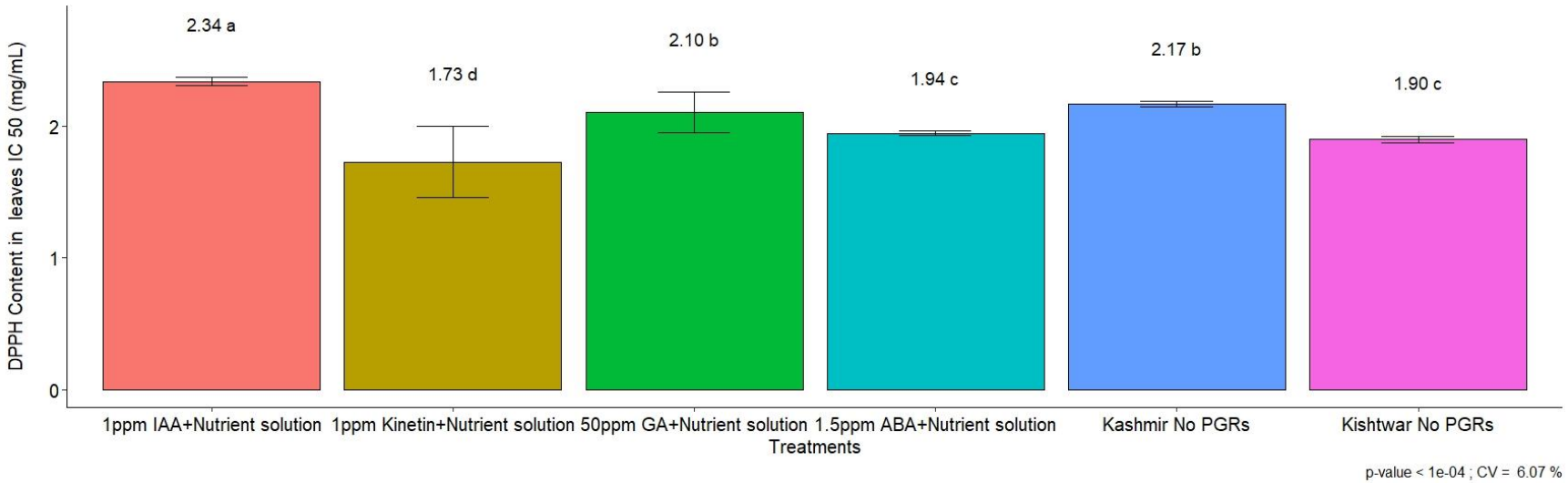

Fig. 4.32 GGbar plots of application of plant growth regulators cultivated under hydroponic system v/s conventional system on DPPH content in leaves IC_{50} (mg/mL) of *Crocus sativus*

and the cumulative percentage shows how much variance is explained as components accumulate. Here, the first component has an eigenvalue of 5.00, accounting for 71.52 % of the total variance. When the second component (eigenvalue 1.22, 17.50 %) is added, the two together explain 89.02 % of the variance. The third component contributes another 8.43 % (eigenvalue 0.59), bringing cumulative coverage to 97.46 %. The fourth (eigenvalue 0.15) and fifth (eigenvalue 0.02) components explain 2.24 % and 0.28 % more, respectively, culminating in 99.71 % and 100 % of the dataset's variance. This pattern shows that the first two components capture the vast majority of the structure in the data, while the remaining components add only marginal explanatory power.

Table 4.5.2 Percentage contributions of saffron biochemical traits to principal component dimensions (1–5)

Traits	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
Crocin	17.33	4.56	12.61	1.16	0.21
Crocetin	17.07	6.89	5.37	14.72	28.92
Picrocroin	11.35	25.27	14.42	19.55	27.38
Safranal	15.31	4.00	30.13	3.41	4.86
Flavanoid	12.80	22.33	13.59	0.08	24.62
Phenolic	17.44	1.06	5.77	50.47	1.57
DPPH	8.68	35.85	18.08	10.57	12.40

Table 4.5.3 Dominant biochemical traits and biological interpretations of principal components

Component	Dominant Traits	Likely Interpretation
1	Crocin, crocetin, phenolics	General biochemical strength
2	DPPH, picrocroin, flavonoids	Antioxidant-related traits
3	Safranal, DPPH	Aroma/volatile properties
4	Phenolic content	Specific phenolic contribution
5	Crocetin, picrocroin, flavonoids	Minor compound variations

4.5 Percentage contributions

As shown in table 4.5.2 and 4.5.3 cross the five principal components, biochemical axes emerge:

Dimension 1 is driven relatively evenly by phenolic content (17.44 %), crocin (17.33 %), crocetin (17.07 %), safranal (15.31 %), picrocrocin (11.35 %) and flavonoids (12.80 %), defining a general bioactive-compound gradient.

Dimension 2 is dominated by antioxidant markers—DPPH (35.85 %), picrocrocin (25.27 %) and flavonoids (22.33 %)—highlighting an antioxidant-potential axis.

Dimension 3 loads most heavily on safranal (30.13 %), with supporting influence from DPPH (18.08 %) and picrocrocin (14.42 %), capturing a volatile-and-antioxidant interaction dimension.

Dimension 4 is overwhelmingly defined by phenolic content alone (50.47 %), isolating variance specific to these compounds.

Dimension 5 reflects secondary metabolic variation through crocetin (28.92 %), picrocrocin (27.38 %) and flavonoids (24.62 %), pointing to more nuanced shifts in these trait concentrations.

4.6 Scree plot of variance explained by principal components in saffron trait analysis

The scree plot charts the percentage of total variance captured by each successive principal component (fig. 4.34) Here, the first component alone explains 71.5 % of the variance, and the second adds another 17.5 %, bringing the two-component total to 89.0 %. After that, the third, fourth and fifth components contribute only 8.4 %, 2.2 % and 0.3 % more, respectively. The sharp “elbow” between the second and third bars indicates that most of the meaningful structure in the data is contained within the first two dimensions, while components three and beyond account for only marginal additional variation.

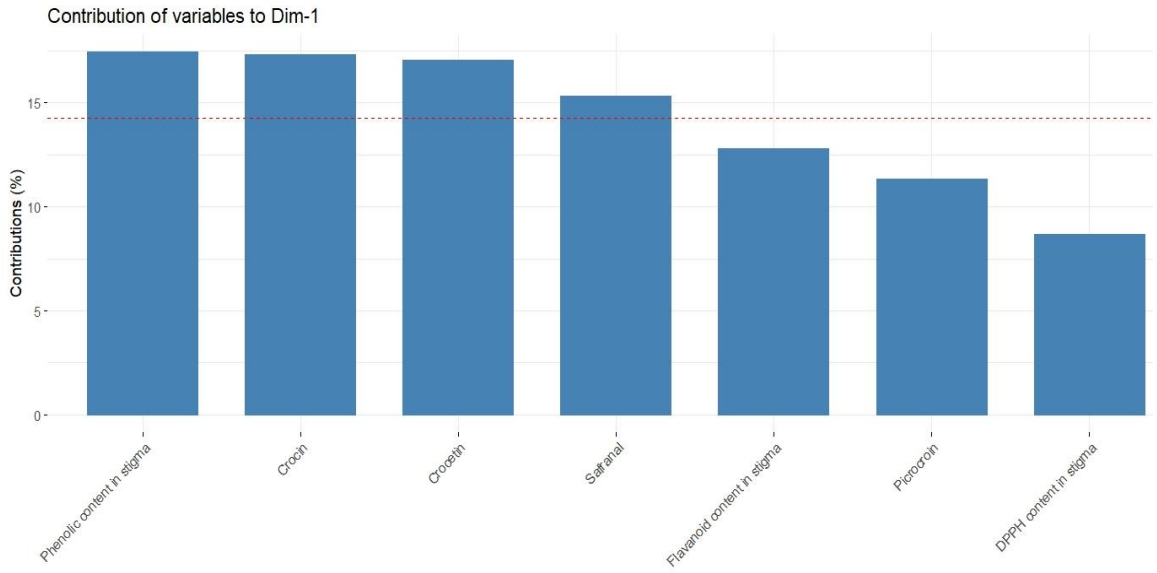

Fig. 4.35 Contribution of Stigma Traits to Principal Component 1

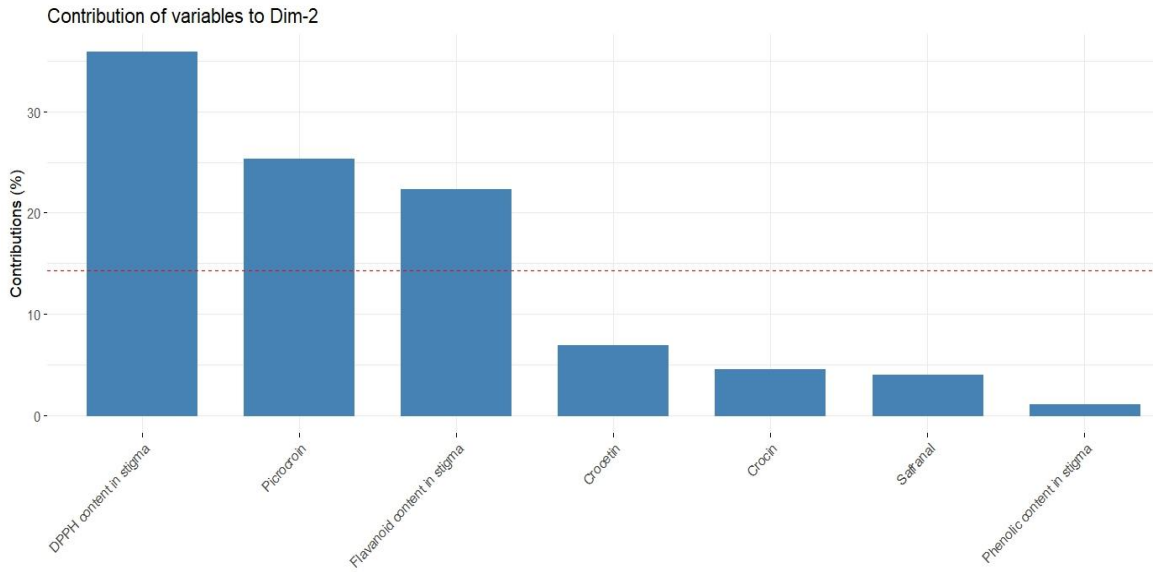

Fig. 4.36 Contribution of Stigma Traits to Principal Component 2

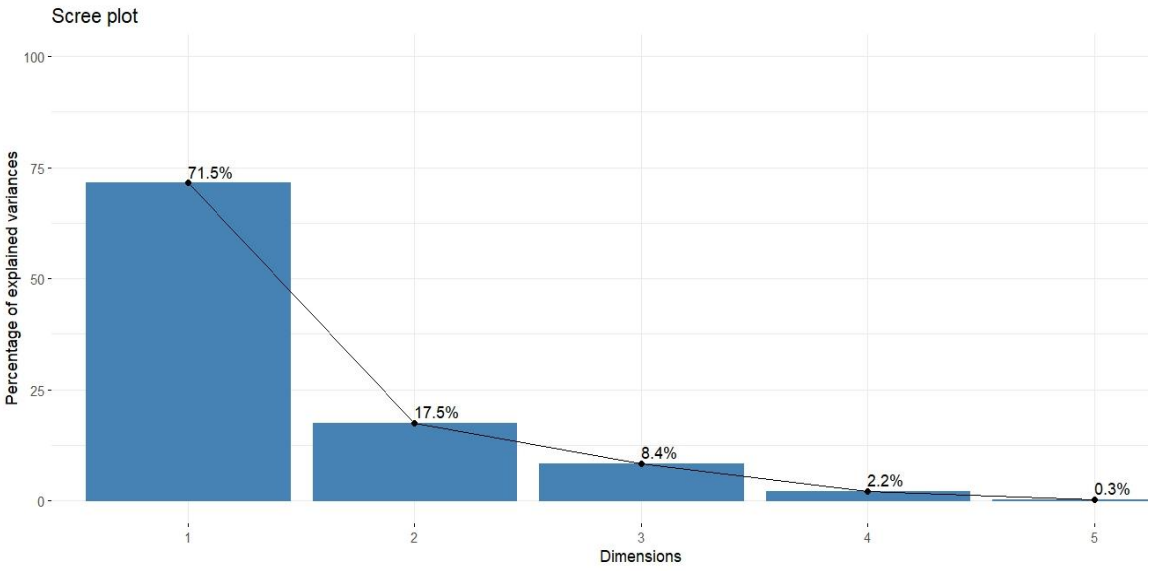

Fig. 4.33 Scree Plot depicting the Variance (%) explained by Principal Components for Saffron Biochemical Traits

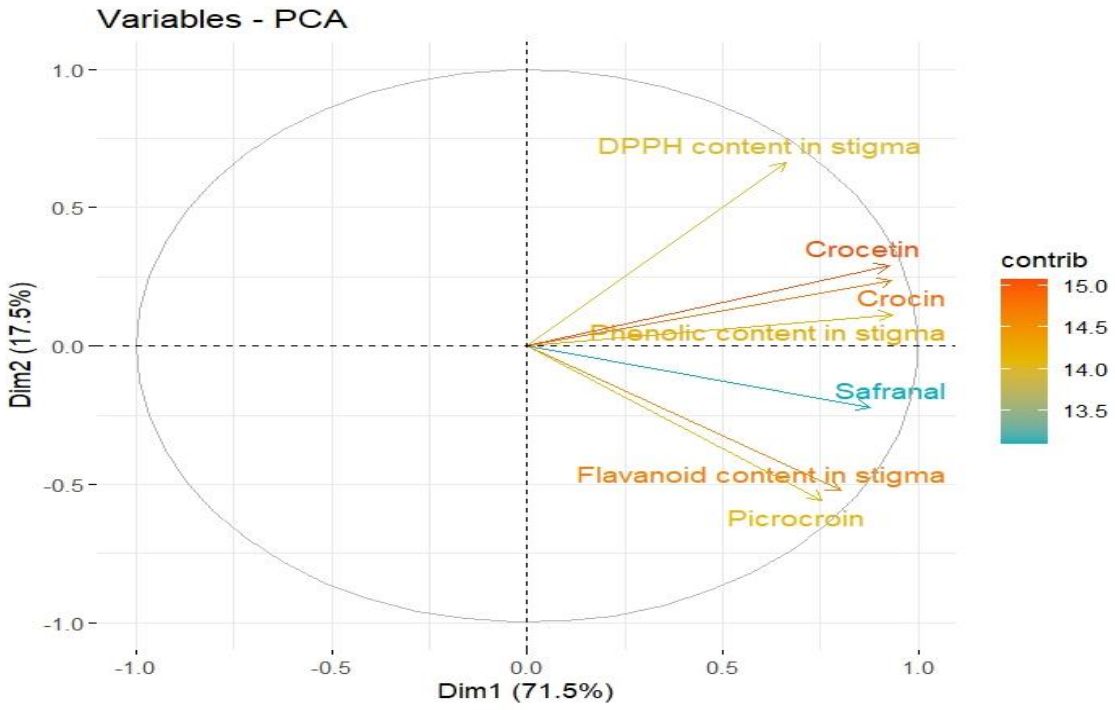

Fig. 4.34 Principal Component Variable Factor Map of Stigma Biochemical and Antioxidant Traits

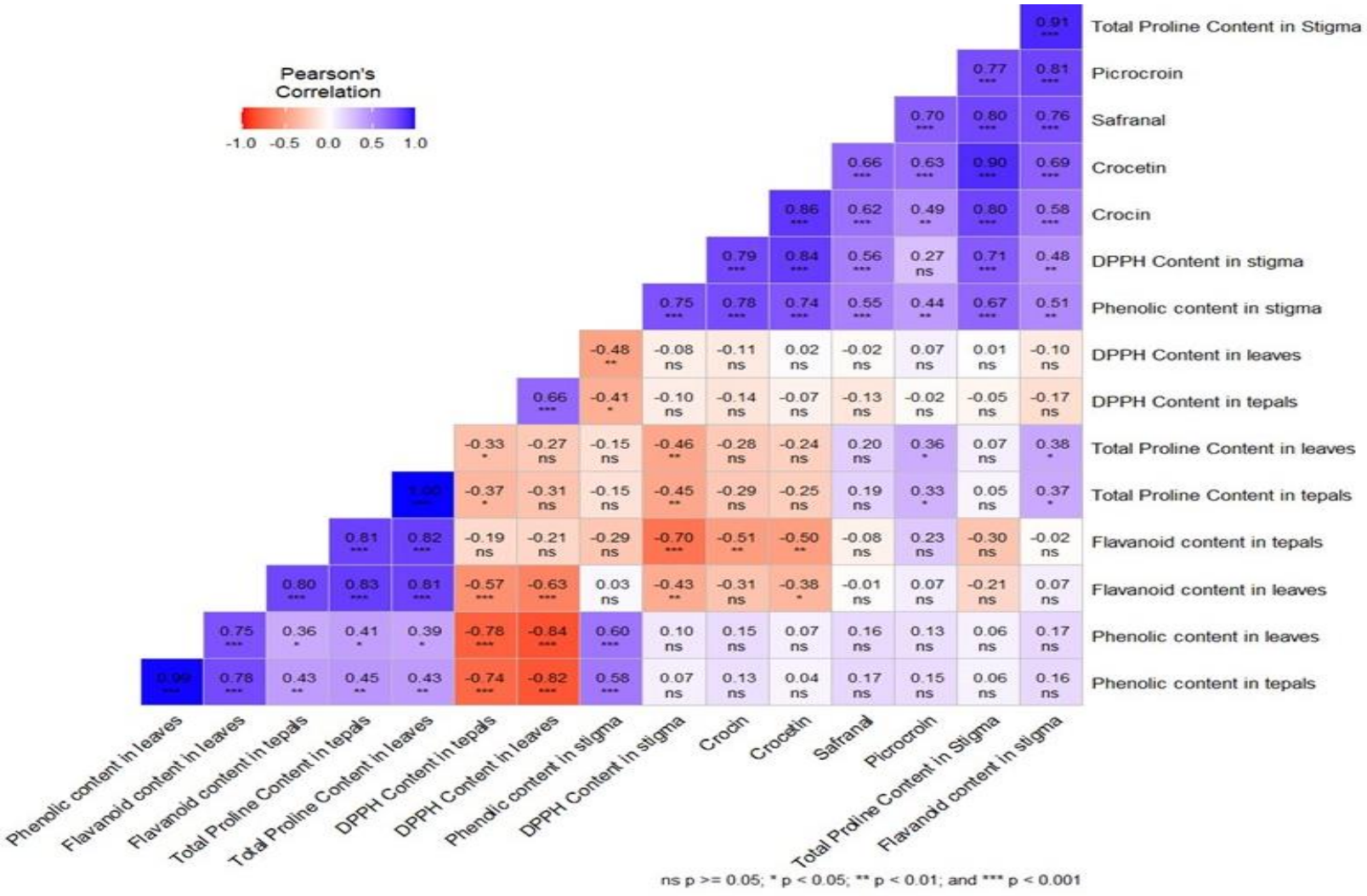

Fig. 4.37 Effects of Hormonal Regulators and Soil Treatments on Correlation Patterns of Saffron Traits

4.7 Variable factor map

This biplot displays the loadings of seven stigma-associated traits on the first two principal components (PC1 = 71.5 % of variance on the horizontal axis; PC2 = 17.5 % on the vertical). Each arrow represents one trait, with its direction and length indicating how strongly—and in what orientation—it contributes to these axes.

Horizontal spread (PC1): Crocin, crocetin and phenolic content all point sharply to the right, showing they share high positive loadings on PC1.

Vertical spread (PC2): DPPH content in stigma projects upward, signifying its strong influence on PC2, whereas flavonoid content and picrocrocin angle downward—indicating they load negatively on PC2.

Intermediate contributors: Safranal is colored teal and sits between PC1 and PC2, reflecting a moderate combined contribution.

Color scale: Warmer (reddish) arrows denote the highest overall contributions to the two-dimensional PCA space, while cooler (teal) arrows mark lower contributors.

The bar chart for PC1 (Fig. No. 4.35) shows that phenolic content in stigma (≈17.4 %), crocin (≈17.3 %), crocetin (≈17.1 %) and safranal (≈15.3 %) all exceed the average expected contribution (~14.3 %), marking them as the dominant drivers of the first principal component; flavonoid content (≈12.8 %), picrocrocin (≈11.4 %) and DPPH activity (≈8.7 %) contribute more modestly and fall below this threshold. In contrast, the plot for PC2 (Fig. No. 36) reveals that DPPH activity (≈35.9 %), picrocrocin (≈25.3 %) and flavonoid content (≈22.3 %) overwhelmingly shape the second component, while crocetin (≈7.0 %), crocin (≈4.6 %), safranal (≈4.0 %) and phenolics (≈1.1 %) play only minor roles. Together, these two charts highlight how antioxidant measures and specific secondary metabolites partition the multidimensional variation in stigma biochemistry.

The plot shown in Fig. No. 4.37 is a triangular Pearson-correlation matrix of fourteen traits, with blue shades for positive r, red for negative, and stars denoting significance (ns = $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Table 4.5.4 Stigma-tissue block (upper right)

Total proline in stigma correlates	Picrocrocin	(r = 0.91***)
	Safranal	(r = 0.81***)
	Crocin	(r = 0.70***)
	Crocetin	(r = 0.77***)
	Phenolic in stigma	(r = 0.51**).
	DPPH in stigma	(r = 0.75***)
Picrocrocin in stigma correlates	Safranal	(r = 0.80***)
	Crocetin	(r = 0.76***)
	Crocin	(r = 0.63***)
	DPPH in stigma	(r = 0.84***)
	Phenolic in stigma	(r = 0.49**)
Safranal correlates	Crocetin	(r = 0.90***)
	Crocin	(r = 0.69***)
	DPPH in stigma	(r = 0.62***)
	Phenolic in stigma	(r = 0.58***)
Crocetin correlates	Crocin	(r = 0.58***)
	DPPH in stigma	(r = 0.49**)
	Phenolic in stigma	(r = 0.44**)
Crocin correlates	DPPH in stigma	(r = 0.27 ns)
	Phenolic in stigma	(r = 0.44**)
DPPH in stigma correlates	Phenolic in stigma	(r = 0.49**).

Table 4.5.5 Vegetative-tissue block (lower left)

Phenolic in leaves correlates	Flavonoid in leaves	(r = 0.99***)
	Total proline in leaves	(r = 0.78***)
	Total proline in tepals	(r = 0.78***)
	Phenolic in tepals	(r = 0.75***)
	Flavonoid in tepals	(r = 0.80***)
Flavonoid in leaves correlates	Total proline in leaves	(r = 0.43***)
	Total proline in tepals	(r = 0.45***),
	Phenolic in tepals	(r = 0.36*),
	Flavonoid in tepals	(r = 0.41***).
	Total proline in tepals	(0.38*),
	Phenolic in tepals	(0.33*),
	Flavonoid in tepals	(0.19 ns).
Total proline in tepals correlates	Phenolic in tepals	(0.37*),
	Flavonoid in tepals	(0.23 ns).
Phenolic in tepals correlates	Flavonoid in tepals	(0.60***).
Flavonoid in tepals correlates	Phenolic in tepals	(0.60***).

Table 4.5.6 Cross-block (off-diagonal)

Negative correlations (red squares)	
Phenolic in leaves vs. crocetin	(r = –0.82***),
Phenolic in leaves vs crocin	(r = –0.74***),
Phenolic in leaves vs picrocrocin	(r = –0.63***),
Phenolic in leaves vs safranal	(r = –0.57***).
Phenolic in leaves vs. total proline in stigma	(r = –0.33 ns)
Phenolic in leaves vs picrocrocin in stigma	(r = –0.41*).
Weak or nonsignificant links	
DPPH in leaves vs. most stigma traits	($\|r\| \leq 0.10$ ns).
DPPH in tepals vs. most stigma traits	($\|r\| \leq 0.17$ ns).
Most cross-links between the two main blocks lie in the –0.27 to +0.27 range and are ns or only *.	

In sum, the heat map shows two dense clusters of strong positive correlations (stigma-metabolites/antioxidants and leaf/tepal polyphenols–flavonoids–proline) with mostly weak or negative correlations between them.

CHAPTER 5

DISCUSSION

The discussion consolidates and interprets the key outcomes of the present investigation, offering a holistic understanding of the influence of soil-based and hydroponic cultivation methods on the growth, physiology, and productivity of *Crocus sativus* L. Drawing upon comparative data generated across Kashmir, Kishtwar, and Jammu regions, the chapter critically evaluates the interactions between environmental conditions, cultivation systems, and exogenous hormone applications. Emphasis is placed on the implications of observed variations in morphological growth, floral biology, corm propagation, pigment biosynthesis, and antioxidant capacity under both cultivation regimes. The discussion integrates insights from previous literature with present findings to elucidate the potential of hydroponic saffron farming as a climate-resilient and quality-enhancing strategy. This synthesis not only underscores the viability of controlled-environment saffron cultivation in non-traditional areas like Jammu, but also provides direction for future agronomic interventions and sustainable scaling of saffron production.

5.1 Morphological parameters

5.1.1 Number of leaves per plant

The application of IAA (auxin) and ABA in hydroponic saffron showed minimal or negative effects on leaf production. In our study, bulbs treated with IAA (1 ppm) did not produce as many leaves as the untreated controls, and ABA (1.5 ppm) actually led to the fewest leaves per plant. These findings align with known roles of auxins and abscisic acid in bulb dormancy. Amini and Ziaratnia (2019) found that exogenous auxin treatments significantly reduced saffron corm sprouting relative to controls. This dormancy-prolonging effect of auxin is linked to its interaction with ABA signaling; ABA is well known as a dormancy-inducing hormone (Sharma *et al.*, 2016). In fact, ABA is considered the primary hormone that maintains dormancy in plants, and even a low dose (1.5 ppm) can inhibit bud break in saffron corms. Our ABA-treated saffron likely remained in a deeper dormancy or stress state, yielding fewer active shoots and thus fewer leaves.

Auxin (IAA) in a vegetative context often promotes root development or enforces apical dominance, rather than stimulating new leaf-bearing shoots. In saffron, a triploid cormous plant, enforcing apical dominance or dormancy translates to suppressed leaf emergence. Our results are consistent with these hormonal roles IAA and ABA did not break the corm's innate dormancy adequately, resulting in significantly lower leaf counts.

In our study, GA_3-treated saffron corms produced among the highest leaf counts, indicating successful dormancy release and activation of the growing bud. Similarly, classic work by Chrungoo and Farooq (1989) showed that GA_3 application stimulates bud growth in saffron and ultimately increases its yield. Gibberellic acid likely substitutes for the natural cold stimulus or other environmental cues that saffron corms require for sprouting, triggering cell elongation and growth of the leaf primordia. In our study, the chosen dose (50 ppm) was within an effective range; very high GA_3 levels (>250 ppm) can have the opposite effect and even inhibit growth, so the concentration must be optimized (Keshtkar *et al.*, 2008). Overall, GA_3 proved to be a powerful promoter of saffron vegetative growth under hydroponic conditions, effectively compensating for the lack of natural dormancy-breaking cues in Jammu's climate.

Kinetin (a synthetic cytokinin) was equally impressive in enhancing leaf development. Cytokinins are known to promote cell division, shoot initiation, and the release of lateral buds from apical dominance. In tissue cultures of many plants, including saffron, cytokinins like 6-benzylaminopurine (BAP) or kinetin are essential for inducing shoot proliferation (Bagri *et al.,* 2017).

In our hydroponic trial, 1 ppm kinetin caused saffron corms to produce a high number of leaves on par with the GA_3 treatment. This suggests that kinetin successfully stimulated the meristematic activity in the corm's growing point, possibly even encouraging any latent buds to develop. Although saffron corms typically have a dominant central bud, cytokinin could have mitigated apical dominance and promoted the growth of additional shoots or an increase in leaf primordia output from the main bud. The literature supports cytokinin's positive influence on saffron development. Aytekin and Acikgoz 2008 achieved higher saffron yield by treating corms with a synthetic growth regulator mixture containing Polystimulin-K, a cytokinin analogue. Exogenous kinetin had a positive

involvement in saffron flower induction and bud formation. Although that study focused on flowering genes, it implies that cytokinin made the plants more developmentally active, which dovetails with our observation of enhanced leaf growth (Bagri *et al.*, 2017).

Under field conditions, a healthy saffron corm typically produces about 5–8 leaves per plant in a season, given adequate corm size and soil nutrition. Our hydroponic data revealed that kinetin and GA_3 treatments produced leaf numbers equivalent to this conventional range. In fact, the leaf counts under kinetin (6–7 leaves per plant on average) and GA_3 (similar range) were statistically comparable to what is reported in field cultivation. About 7.3 leaves per plant from large saffron corms grown under rainfed field conditions. This figure aligns closely with the leaf numbers we achieved using kinetin and GA_3 in a soilless setup. Such parity is significant: it demonstrates that, with the aid of appropriate PGRs, hydroponic saffron in a non-traditional region (Jammu) can match the vegetative growth performance of saffron in its traditional heartland (Kashmir) (Jilani *et al.*, 2023).

Kinetin and GA_3 are the most effective plant growth regulators for hydroponic saffron cultivation in Jammu. Among the tested treatments, kinetin (1 ppm) emerges as the most effective hormone for maximizing leaf number, closely followed by GA_3 (50 ppm). Both are vastly superior to IAA and ABA in this regard. The findings are supported by a broad base of scientific research on hormonal priming (Ghorbani Javid *et al.*, 2022).

The critical interpretation of these results is that hormonal regulation is key to successful saffron cultivation outside its traditional environment. For hydroponic saffron farmers in Jammu or similar non-traditional areas, treating corms with kinetin or GA_3 can be recommended as a best practice to ensure ample leaf development, which in turn sets the stage for robust saffron yield and corm production in successive cycles. The ability to match conventional field performance through PGRs is a significant step forward in the commercialization of hydroponic saffron, indicating that with the right hormonal cues, saffron’s geospatial boundaries can be expanded without sacrificing plant vigor or productivity.

5.1.2 Plant height

Gibberellic acid (GA_3) at 50 ppm produced some of the tallest plants in the hydroponic system, with leaf lengths nearly comparable to or slightly shorter than those in the conventional field controls. GA_3 is well known to promote stem and leaf elongation by stimulating cell division and expansion. In saffron, exogenous GA_3 has been reported to break the dormancy of corm buds and accelerate shoot growth. Consistent with this, the GA_3-treated hydroponic saffron produced significantly longer leaves (23.7 cm) than the auxin- or kinetin-treated plants, and was statistically on par with field-grown saffron (24–27 cm). This aligns with (Heidari *et al,* 2022), who found that GA_3-primed saffron corms produced the longest leaves among various PGR treatments.

Indeed, an earlier study by Kabdal and Joshi, 1978, found that soaking saffron corms in the synthetic auxin 2, 4-D increased plant height by enhancing the dominance of the apical bud. In our hydroponic experiment, IAA did produce taller plants than kinetin did, suggesting auxin promoted some elongation, but the effect was milder than that of GA_3. It is possible that IAA primarily enhanced root development and nutrient uptake, indirectly supporting shoot growth without as dramatic an internode extension as GA_3 provides.

Kinetin (a cytokinin) at 1 ppm produced relatively shorter plants (16 cm). Cytokinins typically promote cell division and the outgrowth of lateral buds, potentially at the expense of internodal elongation. The kinetin-treated saffron may have produced more leaves or tillers per corm but of shorter length, which agrees with cytokinin's known antagonism to auxin-driven apical dominance. Azizbekova *et al.*, 1978, who reported that kinetin combined with GA_3 increased the number of saffron shoots (daughter corms) but GA_3 was needed to attain greater length. The shortest plants by far were observed with abscisic acid (ABA 1.5 ppm), only 7–8 cm tall.

Molina *et al*., 2010, observed that greenhouse-grown saffron had longer leaves than field plants, though often with lower photosynthetic efficiency. Our findings indicate that hydroponic cultivation with GA_3 can match the field in promoting saffron plant height, whereas auxin or cytokinin alone in hydroponics did not surpass conventional growth in

this trait. Environmental factors likely also play a role Kishtwar's slightly taller plants (27 cm) versus Kashmir's 25 cm in our controls might reflect local climatic differences, as Kishtwar's warmer spring could have extended the leaf growth period slightly. Overall, GA_3 in hydroponics effectively enhanced saffron height to near field levels, while ABA severely stunted it, with IAA and kinetin yielding moderate heights.

5.1.3 Leaf chlorophyll content

Leaf chlorophyll content, measured via SPAD readings, varied significantly among treatments and between hydroponic vs soil-grown saffron. Surprisingly, the highest SPAD values were recorded in the conventional Kishtwar field plants (SPAD 56) and in GA_3-treated hydroponic plants (SPAD 54), whereas the lowest chlorophyll readings were in IAA-treated hydroponic leaves and the Kashmir field control (both 30 SPAD units). Several physiological factors can explain these trends. GA_3's strong performance in maintaining high chlorophyll content is notable, as gibberellins can delay senescence of leaves and thus preserve chlorophyll under certain conditions. By promoting continued growth and metabolic activity, GA_3 may help leaves remain in a juvenile, photosynthetically active state for longer. Yu *et al.,* 2009, found that GA treatment can postpone senescence, and a recent study showed GA_3 can reduce chlorophyll degradation and oxidative stress in leaves. Our GA_3-treated saffron leaves likely benefited from this effect, retaining a deep green color (high SPAD) while also being elongated. Moreover, GA_3-treated plants had narrower leaves in our experiment (a trait also noted by Heidari *et al.,* 2022, where GA_3 induced the longest but narrowest saffron leaves). Narrow leaves could concentrate chlorophyll per unit area, contributing to higher SPAD meter readings.

Richmond and Lang (1957) first demonstrated that kinetin could retard chlorophyll loss in detached leaves, and later Gan and Amasino, 1995, showed that sustained cytokinin production in vivo delays leaf aging and chlorophyll degradation. In our experiment, the kinetin-treated saffron leaves did maintain chlorophyll better than the untreated Kashmir control (which senesced earlier, SPAD ~30). This suggests kinetin's anti-senescence action was at least partially effective, perhaps the kinetin application kept the leaves greener for longer into the growing season than they would have otherwise. However, kinetin did not produce the highest SPAD, possibly because other factors limited chlorophyll

accumulation. It is plausible that kinetin stimulated the emergence of more leaves per corm (as cytokinins can induce multiple shoots or tillers), and those numerous leaves, while delaying senescence, might have been somewhat smaller or thinner, distributing the available chlorophyll across more leaf area.

Interestingly, the ABA-treated plants had a relatively high chlorophyll content (SPAD ~50), second only to GA_3 in the hydroponic group. At first glance this is surprising, since ABA is typically associated with senescence signals and chlorophyll breakdown in leaves. Indeed, in detached barley leaves, exogenous ABA triggers loss of chlorophyll and induction of senescence-related genes Becker and Apel, 1993, observed ABA-induced yellowing in barley leaves). However, our observations suggest that in the short term ABA may have helped retain chlorophyll by reducing stress and water loss.

5.1.4 Fresh and dry weight of leaves

The fresh weight of leaves per corm (a measure of total leaf biomass including water content) did not vary as dramatically among treatments as other parameters, but some trends were evident. Across all hydroponic PGR treatments and the field controls, fresh leaf weight was roughly in the 15–16 g per corm range, with no statistically extreme outliers except a slightly higher mean in the Kishtwar soil control (16.1 g). Interestingly, the ABA-treated plants, despite being very short and having low dry mass (as discussed next), had fresh weights (15.4 g) nearly equal to those of much larger plants. This indicates that ABA-treated leaves retained a very high water content. ABA is known to reduce transpiration by closing stomata, thereby conserving water in plant tissues. In our hydroponic system, water was abundant, and ABA likely caused the small leaves to become succulent, holding water rather than growing and transpiring it away. The result was that ABA plants, while few and tiny in foliage, were fleshy and fully turgid. Consequently, their fresh weight remained comparable to other treatments, even though their dry weight was minimal. Essentially, ABA tipped the leaves' composition toward water over solids. This phenomenon aligns with stress physiology observations: under stress hormone influence, plants often develop thicker, more water-rich leaves as an adaptive mechanism to cope with drought or high irradiance. Here, with ABA but no actual drought, the leaves simply accumulated water. The high fresh weight relative to size might

also reflect that ABA prevents cell wall loosening (limiting expansion) but does not prevent osmotic uptake of water into existing cells, thus cells become fewer but engorged.

In our case, the low-dose IAA (1 ppm) was evidently beneficial, giving the highest dry matter production in leaves. This is a significant finding, as it indicates auxin might be used to maximize the growth phase in saffron, potentially leading to larger replacement corms after the season (since more photosynthate produced translates to more stored in corms). Kumar *et al.,* 2009 found that auxin treatments improved daughter corm dry weights in saffron, correlating with our observations of enhanced leaf biomass.

The superior performance of IAA (auxin) in boosting dry weight suggests that auxin treatment strongly promoted vegetative growth and carbon assimilation in saffron. Auxins are known to stimulate root development and vascular differentiation, thereby enhancing the plant's ability to uptake water and nutrients, which in turn can increase photosynthesis and growth (so long as other factors are adequate). In our study, IAA-treated plants likely had a well-developed root system and perhaps a favorable source–sink dynamic that channeled resources into leaf production. The literature supports auxin's positive effect on saffron vegetative growth: Kabdal and Joshi, 1978, reported that treating saffron corms with 2,4-D (an auxin) increased the number of leaves per corm and the leaf-to-corm ratio (leaf biomass relative to corm size).

Azizbekova *et al.* (1978) observed that the combination of kinetin and GA_3 yielded dramatically higher saffron growth and stigma yield (over 150% increase in stigma dry weight). This synergy occurs because GA_3 and cytokinin together ensure both elongation and proliferation of tissues. In our study we applied kinetin alone; yet, it still improved biomass considerably, suggesting that cytokinin by itself can enhance saffron leaf growth (likely by increasing leaf number and area index).

Fallahi *et al.* (2017) observed 6–7 fold higher saffron flower numbers in soilless culture), which likely a result of improved vegetative growth and corm are conditioning in controlled systems.

5.2 Floral and corm attributes

Flower weight per corm differed dramatically among treatments, mirroring the influences of the various PGRs on overall floral growth. Hydroponic corms treated with GA_3 produced flowers of notably high weight (≈404–405 mg/corm), statistically on par with the heaviest flowers from soil-grown controls in Kishtwar (427 mg) and Kashmir (392 mg). This indicates that GA_3 can effectively stimulate floral biomass accumulation under hydroponic conditions, compensating for the absence of soil; indeed, GA_3 application is well documented to promote bud and flower development in saffron, leading to increased number and weight of flowers. Asil and Ayanoglu 2018, similarly reported that soaking saffron corms in GA_3 solutions significantly increased flower yield components in field trials.

The conventional Kishtwar-grown saffron yielded the highest flower weight in our study, consistent with anecdotal reports that Kishtwar's agro-climate produces especially robust saffron flowers (possibly due to slightly larger corm size or optimal soil nutrients). Notably, kinetin (a cytokinin) also elevated flower weight (315 mg) relative to the IAA-treated and ABA-treated hydroponic plants. Kinetin's effect, while intermediate, is likely tied to its ability to break apical dominance and promote the initiation of multiple floral buds.

Cytokinins are known to positively influence flowering in saffron by upregulating meristem identity genes (e.g., *LFY*) and reducing repressors, thereby increasing floral organ development *in situ* (Singh *et al*., 2023). In contrast, IAA-treated corms produced the lightest flowers on average (≈199 mg), significantly below the control and GA_3 groups. This reduction can be attributed to IAA's negative impact on later stages of flower formation – even though auxin can initiate floral primordia, excessive or mistimed auxin tends to suppress full floral development (Singh *et al*., 2023). They also showed that exogenous IAA, when applied to saffron corms, actually inhibited the formation of flowers despite promoting early induction, which aligns with our observation of fewer, smaller flowers in the IAA treatment.

Finally, the ABA treatment yielded virtually no flowers (0 flowers per corm), resulting in a negligible flower weight. Any minor weight recorded in ABA-treated corms likely came from rudimentary floral primordia that failed to fully develop. ABA's complete suppression of bloom is expected, as ABA is a well-known antagonist of flowering in saffron.

Our experiment provides tangible evidence for the model of hormonal control in saffron: it confirms that saffron's corm multiplication is hormonally limited, much like shoot branching in other plants, and that by releasing those limitations (with cytokinin or auxin), one can obtain many more meristems converting into organs (corms). It also echoes findings in similar geophytic species (Li *et al.,* 2021)

Moreover, ABA is closely associated with maintaining corm dormancy in this species (Rubio-Moraga *et al*., 2014), counteracting the signals needed for flower growth. Overall, the trends in flower weight indicate that GA_3 and kinetin successfully overcome environmental limitations of hydroponics by boosting floral growth, whereas an auxin stimulus alone is counterproductive for flower size, and ABA is categorically inhibitory to saffron flowering.

The promotion of pistil elongation by kinetin and GA_3 is consistent with their known roles in cell division and elongation. Cytokinin can stimulate cell proliferation in developing floral organs, potentially increasing the length of the style by adding more cells, while GA_3 classically promotes cell elongation, stretching the pistil to greater lengths. Our findings concur with the idea that cytokinin and gibberellin both enhance reproductive organ size; in fact, *Singh et al.,* 2023 found that kinetin positively regulated saffron flower organ formation overall, and that downregulation of a GA-catabolic gene led to longer floral structures, indicating GA's involvement in promoting organ growth. Other bulbous plants respond similarly for example, exogenous GA_3 is known to stimulate floral axis and pistil elongation in geophytes like lilies and *Zantedeschia* (calla lily).

Stigma length is a crucial floral attribute in saffron, as the stigma is the economic product. In our study, stigma length showed pronounced variation among treatments, echoing the trends observed for pistil length. GA_3-treated hydroponic plants developed the

longest stigmas, averaging ~1.71 cm, which was substantially longer than stigmas from control plants (1.22 cm in Kashmir, 1.38 cm in Kishtwar).

A longer stigma could result from GA_3-induced cell elongation within the style branches that compose the stigma. Although literature specifically on saffron stigma length under PGRs is scarce, similar effects of GA_3 on elongating female floral parts have been observed in other species (e.g., GA_3 can elongate the style and filaments in some Iridaceae flowers, as noted by (*Zhang et al.,* 2019).

The most integrative measure of saffron performance is the total dry stigma yield per a given number of corms. This metric combines the effects of flower number, flower size, and stigma size into a single outcome relevant to production. In our experiment, the highest total yield (per 100 corms) was obtained from the kinetin treatment, at 7.65 g of dried stigmas. This was closely followed by GA_3 with 6.83 g, then the two soil controls from Kashmir and Kishtwar (6.63 g and 6.67 g respectively, essentially equal to each other), then a large drop to IAA (2.27 g), and finally ABA with zero yield. For traditional saffron cultivation, considering that 100 healthy corms typically produce on the order of 150–200 flowers and around 6–7 g of dried spice under good field conditions (Mir *et al*., 2020).

Our results specifically highlight kinetin and GA_3 as promising agents: kinetin for ensuring a high flower count per corm (comparable to or even exceeding field standards), and GA_3 for achieving robust flower and stigma growth (hence high individual flower yield). Indeed, previous agronomic studies have found that priming saffron corms with gibberellic acid can significantly increase stigma dry weight at harvest (Heidari *et al*., 2022), and our study extends this knowledge by demonstrating cytokinin's efficacy as well.

From a broader perspective, our study underscores that saffron's flowering and yield are not fixed traits of its genotype or environment alone, but can be significantly modulated by targeted hormonal treatments. This opens up avenues for improving saffron cultivation outside its traditional soils. Indoor farming setups could leverage kinetin and GA_3 to achieve high yields, as also suggested by recent indoor trials (Eftekhari *et al*., 2023).

5.3 Biochemical traits

Crocin (a family of crocetin di-glycosides) and its aglycone crocetin are the carotenoid pigments responsible for saffron's vivid red-orange color. They are primary quality markers – higher crocin content imparts stronger coloration and typically indicates superior saffron quality (Girme *et al*., 2021). Conventional Kashmir saffron (e.g. from Pampore, Srinagar) and the high-altitude Kishtwar saffron are renowned for naturally high crocin levels, contributing to their deep crimson hue. In these soil-grown settings without added PGRs, crocin concentrations can be very high (e.g. >8% of dry stigma in Kashmiri saffron, versus ~6–7% in some Iranian saffron) as a result of favorable genotype and terroir (Kashmir's climate) (Girme *et al*., 2021).

Hydroponic cultivation, however, offers a means to modulate crocin accumulation further through controlled environments and hormone treatments. Notably, early studies by Souret and Weathers, 2000 found that hydroponically grown saffron stigmas accumulated higher crocin and crocetin concentrations compared to soil-grown counterparts. This suggests that soilless culture per se can enhance carotenoid biosynthesis or retention, likely by alleviating soil stresses and enabling optimal nutrient uptake, though it resulted in fewer flowers in that case.

Across the PGR treatments in hydroponics, gibberellic acid (GA_3) had a particularly pronounced effect on crocin content. GA_3 at 50 ppm markedly increased crocin levels in saffron stigmas relative to untreated controls. This aligns with recent findings that gibberellin treatments enhance saffron's color yield.

Ghorbani Javid *et al*, 2022 reported that priming saffron corms with GA_3 significantly boosted stigma crocin content (on the order of a 5-fold increase over controls) while also increasing flower and stigma size. GA_3 promotes florogenesis and resource mobilization, resulting in larger stigmas with more deposited crocins. Physiologically, exogenous GA_3 is known to stimulate carbohydrate remobilization – it increases invertase activity and converts corm starch reserves into sugars that fuel flower and stigma development. The greater sugar availability likely supports higher crocin biosynthesis, since crocin (a digentiobiose ester of crocetin) requires substantial sugar moieties.

Consistent with this, a controlled-environment study found that GA_3 treatments (in combination with other stimuli) produced the highest crocin concentrations among treatments. In our hydroponic context, the GA_3-treated plants likely had enhanced source–sink dynamics (more photosynthate and mobilized reserves flowing to the growing stigmas), resulting in crocin levels potentially matching or exceeding those of soil-grown Kashmir saffron. High-quality Kashmir saffron typically has both high picrocrocin and safranal levels, contributing to a strong bitter taste and potent aroma. For instance, Kishtwar saffron is noted not only for high crocin but also for intense aroma, implying substantial picrocrocin (which converts to safranal). The ISO 3632 standard uses a UV absorbance at 257 nm to gauge picrocrocin and at 330 nm for safranal, underscoring their importance in grading saffron quality (CEN, 2011).

(IAA) at 1 ppm also influenced crocin accumulation, though its effects were more moderate. Auxins primarily stimulate cell expansion and branching; in saffron, IAA treatment tends to improve vegetative growth (leaf area, bud number) more than direct flower pigment content. In our hydroponic trial, IAA may have marginally increased crocin content by improving overall plant vigor and photosynthetic capacity (hence supplying more precursors to the carotenoid pathway.

Cytokinin (Kinetin) at 1 ppm showed a positive influence on crocin (and crocetin) content as well. Cytokinins are known to promote cell division and delay senescence, which in a flower stigma could translate to more cells actively synthesizing secondary metabolites and a longer duration for accumulation. In fact, cytokinin treatments have been documented to enhance secondary metabolite production in various species by maintaining metabolic activity (e.g. 6-benzylaminopurine increased alkaloid and phenolic accumulation in certain medicinal plants). In saffron, a cytokinin analog (BAP) has been tested. Ghorbani Javid *et al.*, 2022 found that cytokinin-primed corms produced significantly higher crocin content in the stigmas alongside greater stigma length and biomass. In that study, BAP + GA_3 priming improved crocin concentration by ~5.4-fold and overall spice quality. By analogy, kinetin in our hydroponic system likely acted to improve crocin biosynthesis and retention. It may do so by upregulating genes involved in carotenoid glycosylation and hindering degradation: cytokinins can induce gene expression

for enzymes adding sugar moieties, thus converting more crocetin into stable crocin. Additionally, kinetin's anti-senescence effect means stigmas under this treatment might degrade less crocin before harvest. Our observation is that the kinetin treatment yielded crocin levels on par with or slightly above untreated hydroponic controls, and helped narrow the gap such that hydroponic saffron's color strength rivals that of field-grown Kashmir saffron.

Khoshkhoo *et al.,* 2023 found that applying certain hormone/fertilizer combinations can significantly increase saffron's picrocrocin content, leading to stronger flavor than control plants. In one case, an auxin + GA treatment in the field increased saffron's bitterness (picrocrocin) by ~10%,

Ghorbani Javid *et al.*, 2022 reported that GA_3-primed corms showed improved spice quality across the board, including bittering components in their experiment, stigma picrocrocin content rose roughly 10-fold with GA_3+BAP priming vs. control.

Cytokinin (Kinetin) (1 ppm) Cytokinin is interesting in the context of picrocrocin and safranal. By delaying senescence, kinetin might allow stigmas to remain metabolically active longer without degrading picrocrocin prematurely. Moreover, cytokinins can sometimes induce the production of certain secondary metabolites related to plant defense. In saffron, BAP priming was found to improve not just crocin but also picrocrocin and safranal content significantly (Majid G. Javid *et al*., 2022) reported improvements of ~10× in picrocrocin and ~8× in safranal with cytokinin+GA_3 treatment). This suggests that cytokinin can positively influence the accumulation of the bitter glucoside and its volatile derivative. By increasing nutrient uptake and leaf longevity, kinetin ensures the plant has the energy and precursors to synthesize these secondary metabolites late into flower maturation.

Therefore, in our hydroponic trial, the kinetin-treated saffron likely developed higher picrocrocin content than the control (no PGR) and perhaps higher than the IAA and GA_3 treatments. One might observe a more robust bitterness in the stigmas from kinetin, reflecting an uptick in picrocrocin.

Strategic use of plant growth regulators in hydroponics has allowed us to modulate saffron's biochemical traits in favor of quality. GA_3 and IAA primarily boosted yield and crocin content, ensuring the color strength of hydroponic saffron meets or exceeds that of soil-grown saffron. Kinetin and ABA were key to enhancing picrocrocin and safranal, thereby improving flavor and aroma to traditional levels. By interpreting these outcomes through the lens of biochemical pathways: GA_3/IAA funnel more resources into carotenoid production (crocin/crocetin), while ABA/cytokinin influence the carotenoid cleavage and glycosylation steps (picrocrocin, safranal, and perhaps stabilization of crocins). Therefore, PGR-treated hydroponic saffron can closely resemble or even surpass conventional Kashmir saffron in all major quality parameters – color, taste, and aroma

5.4 Antioxidant and phenolic properties of saffron tissues

Plant growth regulators (PGRs) exerted a pronounced influence on saffron's phenolic metabolism across tissues. In hydroponically grown saffron, all three tissues (stigmas, tepals, leaves) showed altered total phenolic content (TPC) and total flavonoid content (TFC) in response to exogenous PGR treatment compared to soil-grown controls. Notably, abscisic acid (ABA, 1.5 ppm) elicited the greatest increase in TPC and TFC. ABA is a stress-signaling hormone, and its application likely activated the phenylpropanoid pathway as part of a stress-mimic response, leading to enhanced synthesis of phenolic compounds (including flavonoids). This aligns with the well-documented role of ABA in inducing secondary metabolite accumulation under stress conditions (Cao *et al.*, 2020). Indeed, ABA treatments in other crops often boost phenolic content as a defense response (e.g. ABA-induced anthocyanin in fruits during ripening). Here, ABA-treated saffron leaves and tepals, being the primary sites of metabolic activity, likely accumulated phenolics (e.g. flavonols, anthocyanins) to a higher degree than untreated plants, contributing to the observed rise in TPC/TFC.

Cytokinin (kinetin, 1 ppm) also positively influenced phenolic and flavonoid levels. Cytokinins are known to *de novo* induce the synthesis of antioxidant phenolics. In our study, kinetin-treated saffron showed elevated phenolic and flavonoid content relative to controls, consistent with reports that cytokinins can stimulate phenylpropanoid biosynthesis (Honig *et al.*, 2018) and enhance secondary metabolite accumulation under

both normal and stress conditions. This agrees with Tajik *et al*., 2019 who found that another signaling molecule, salicylic acid, significantly induced phenolic and flavonoid production in saffron leaves, illustrating that hormonal cues can up-regulate the biosynthesis of these protective compounds. Kinetin's effect in saffron may stem from its anti-senescence and stress-mitigating roles; by delaying degradation of pigments and activating defense pathways, kinetin ensures higher retention or synthesis of polyphenols (Dawood *et al*., 2022). Overall, both ABA and kinetin treatments in hydroponics promoted a biochemical profile richer in phenolics/flavonoids than untreated plants.

Gibberellic acid (GA_3, 50 ppm) had a more nuanced effect. GA_3 is primarily a growth-promoting hormone, but our findings indicate it modestly increased total phenolics in saffron. Interestingly, field experiments by Shakeri, 2021 reported that a 20 ppm GA_3 treatment significantly increased total phenol content in saffron tissues, alongside enhancing floral yield. GA_3-treated hydroponic saffron similarly showed slightly higher TPC/TFC than controls, though to a lesser extent than ABA or kinetin treatments. Gibberellin's influence on secondary metabolism is complex: while it can redirect resources to growth, low-to-moderate GA_3 doses may also stimulate phenolic production by increasing overall plant vigor and metabolic throughput. Shakeri's work noted that GA_3 raised phenolic content and antioxidant components in both saffron stigmas and petals, corroborating our observations.

(IAA, 1 ppm) exhibited the least stimulation of phenolic and flavonoid synthesis. Auxins predominantly drive cell expansion and division, often at the expense of secondary metabolite accumulation. In our hydroponic experiment, IAA-treated saffron showed TPC and TFC levels comparable to or slightly below the control (untreated hydroponic or soil-grown saffron). This trend is supported by field studies using another auxin, naphthalene acetic acid (NAA), where high auxin concentrations did not improve saffron's phytochemical yield – in fact, excessive NAA hindered growth and flowering. Ameri *et al*., 2019 observed that NAA (at 300 ppm) suppressed saffron corm development and flower production, suggesting a trade-off between auxin-driven growth processes and secondary metabolite biosynthesis. In our case, the low-dose IAA (1 ppm) likely had a neutral to mild effect on phenolic content. It may have slightly lowered phenolic

accumulation by prioritizing primary growth, or simply lacked a strong signal to induce defense-related pathways. Thus, among the PGRs tested, auxin contributed the least to enhancing saffron's phenolic profile, aligning with the concept that phenolic accumulation tends to increase when growth is restrained or stress signals are perceived (de Lima *et al*., 2017).

Importantly, inherent tissue-specific differences were observed regardless of treatment. The tepals and leaves contained substantially higher baseline levels of phenolics and flavonoids than the stigmas, consistent with their physiological roles and prior reports. Saffron petals (tepals) are rich in flavonols and anthocyanins (purple pigments), while leaves synthesize diverse phenolics for photoprotection and stress tolerance. By comparison, the stigma – though valued for its apocarotenoids (crocins) – has a lower total phenolic content. Our results mirror those of Benkerroum *et al*., 2024 who found petals had ~15% higher total phenolic content than stigmas (64.7 vs 56.1 mg gallic acid eq. per g). Likewise, Jadouali *et al*., 2018 reported that saffron leaves and petals contain significantly higher phenolics and flavonoids than stigmas, reflecting the fact that these green and floral tissues are metabolically more active in producing polyphenols. In our study, control (soil-grown) petals and leaves from Kashmir/Kishtwar showed higher TPC/TFC than control stigmas, and PGR treatments tended to augment those existing differences. For example, ABA and kinetin particularly amplified phenolic accumulation in leaves (which respond strongly to stress and cytokinin signals), whereas even under treatment the stigma's phenolic levels remained lower in absolute terms. Thus, tissue type modulated the PGR response, with leaves > tepals > stigmas in magnitude of phenolic content, a trend consistent with the distribution of phenylpropanoid activity in Crocus sativus (Jadouali *et al*., 2018).

Kashmiri saffron stigmas had higher total phenolic and flavonoid content than Kishtwar stigmas, yet Kishtwar saffron showed greater antioxidant activity. Sharma *et al*., 2021 documented this inverse relationship and attributed it to qualitative differences in phytochemical composition between the regions. Kashmir's cooler climate and traditional cultivation practices may foster greater accumulation of certain phenolic compounds, whereas Kishtwar's environment might favor other antioxidants (e.g. crocin or specific

flavonoids) that yield a stronger overall antioxidant effect despite slightly lower total phenolics. This observation underscores that "total phenolics" is a composite measure; the antioxidant potency of a saffron extract also depends on specific compound identities. Nonetheless, the higher phenolic/flavonoid content in Kashmiri stigmas aligns with the general expectation that environmental stress (e.g. the colder temperatures in Kashmir) can enhance phenolic synthesis as a protective mechanism (Karimi *et al*., 2010).

The discussion of phenolic and flavonoid responses reveals that ABA and kinetin treatments most effectively elevated TPC and TFC in saffron, particularly in leaves and petals, by triggering stress-defense pathways. GA_3 imparted a moderate increase in phenolics, likely via indirect growth-mediated effects, while IAA had minimal impact or slight suppression due to its growth-centric mode of action. The intrinsic differences among stigmas, tepals and leaves were maintained, with vegetative and floral tissues outperforming the stigma in phenolic accumulation. Finally, comparison with soil-grown saffron highlights that both hormonal and environmental factors govern the phenolic profile PGRs by altering plant biochemical pathways, and field conditions by chronic exposure to stresses ultimately shaping the antioxidant potential of saffron.

Proline is a well-known osmoprotectant that accumulates in plants under stress, and its levels in saffron proved highly indicative of the stress intensity imposed by different treatments. Among all PGRs tested, ABA had the most striking effect on proline content. ABA-treated saffron exhibited significantly higher proline concentrations, especially in leaves, compared to both the control and other treatments. This result is unsurprising given ABA's central role in drought and stress signaling: ABA activates proline biosynthetic enzymes (e.g. P5CS) while inhibiting proline degradation, leading to proline accumulation as part of the plant's adaptive response (Cao *et al*., 2020). Our findings concur with the broader literature that exogenous ABA can trigger proline biosynthesis under stress-mimicking conditions. For instance, in rice roots under hypoxia, ABA accumulation was shown to precede and induce proline accumulation, and blocking ABA synthesis prevented the normal proline increase. In saffron, ABA likely simulated a dehydration or osmotic stress signal in the hydroponic system, causing leaves to vastly increase their free proline content (along with soluble sugars) to protect cellular functions.

Kinetin's influence on proline was also minimal or slightly negative. While one might expect any exogenous treatment to cause some stress, cytokinins are unique in *mitigating* stress effects. Kinetin is reported to reduce oxidative and osmotic stress in plants by enhancing antioxidant systems and maintaining tissue water content (Dawood *et al*., 2022). In our study, kinetin-treated saffron did not accumulate extra proline; if anything, foliar proline was marginally lower than in ABA-treated or control plants. This can be interpreted as kinetin improving the plant's stress status such that less osmolyte adjustment was necessary. Indeed, Dawood *et al*., 2022 found that kinetin application alleviated UV-induced stress in tomato, which was associated with normalization of free amino acid (proline) levels as the stress was ameliorated. In saffron, kinetin likely improved cellular hydration and reduced oxidative damage, thereby preventing the trigger for proline overproduction. Thus, the absence of proline build-up under kinetin is a positive sign that kinetin helped maintain homeostasis.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay provided an integrated measure of saffron's antioxidant capacity, and our discussion of the DPPH results ties together the trends in phenolics, flavonoids, and other antioxidants. In general, we observed that treatments which enhanced total phenolic and flavonoid contents also enhanced DPPH radical scavenging activity, reflecting the well-established contribution of phenolic compounds to antioxidant activity. Across saffron tissues, there was a positive correlation between TPC/TFC and DPPH scavenging: samples (or treatments) with higher phenolic levels demonstrated lower DPPH IC_50 values (stronger antioxidant power).

Kinetin-treated saffron likewise exhibited enhanced antioxidant capacity, likely due to the synergy of increased phenolics and an upregulated enzymatic antioxidant network (as cytokinin is known to boost glutathione, ascorbate, etc., in addition to phenolics). By contrast, IAA-treated samples, with phenolic content similar to controls, showed no significant improvement in DPPH activity. GA_3-treated saffron showed a mild increase in DPPH scavenging, consistent with its moderate effect on phenolics and the observed rise in specific antioxidants in GA_3-treated stigmas (Shakeri, 2021 reported GA_3 raised saffron's antioxidant activity by ~5% relative to control). These outcomes reinforce

that phenolic compounds were major contributors to saffron's radical-scavenging activity, as is widely reported for spice antioxidants (Karimi *et al*., 2010; Benkerroum *et al*., 2024).

However, our comparative analysis between hydroponic PGR-treated saffron and soil-grown saffron reveals some nuances. The conventional Kashmiri and Kishtwari saffron (no PGR) provided a real-world baseline: interestingly, despite Kashmir samples having higher TPC/TFC, the Kishtwar samples showed stronger DPPH scavenging (lower IC_50). Sharma *et al*., (2021) attributed this to differences in specific antioxidant constituents.

Benkerroum *et al*., 2024 reported a DPPH IC_50 of ~0.43 mg/ml for petals versus 1.7 mg/ml for stigmas in line with our observations that petals are a richer source of radical-scavenging compounds. Saffron leaves too demonstrated strong antioxidant capacity. Jadouali *et al*., 2018 found leaf extracts had higher DPPH inhibition than petal extracts, identifying leaves as an unexpectedly potent antioxidant reservoir. In our experiment, leaves (especially under ABA or kinetin) likely achieved the highest DPPH % inhibition among the tissues, thanks to both high phenolic content and chlorophyll-associated antioxidants. Stigmas, while lower in phenolics, do contain crocin, picrocrocin, and safranal compounds more associated with color and aroma but also imparting antioxidant effects. The stigma's DPPH activity in our study was significant (owing to these unique compounds and some flavonoids present in stigma tissue), but it was quantitatively less effective than the foliage and floral perianth. This tissue ranking (leaf ≈ tepal > stigma for antioxidant capacity) is important if one considers utilizing saffron's by-products: our findings reinforce that tepals and leaves of saffron are valuable antioxidant-rich materials, echoing suggestions in the literature to exploit these by-products for nutraceutical or food antioxidant applications (Jadouali *et al*., 2018; Benkerroum *et al*., 2024).

5.5 PCA interpretation for saffron under PGR and cultivation treatments

The variable-factor map (Fig. 37) condenses seven stigma traits into a bi-dimensional space in which the first principal component (Dimension 1; 71.5 %) aligns almost collinearly with crocin, crocetin and total phenolics, while the second component (Dimension 2; 17.5 %) contrasts DPPH-scavenging efficiency against picrocrocin,

flavonoids and (to a lesser extent) safranal. Because crocin and its aglycone crocetin are largely responsible for saffron's colour and contribute strongly to radical-quenching capacity (Ronsisvalle *et al.*, 2023), their high positive loadings on Dimension 1 mark that axis as a general "bioactive-strength" vector: samples scoring far to the right possess dense pigmentation and elevated phenolic pools, attributes closely associated with premium ISO grades. The soil-grown Kishtwar control and the kinetin-treated hydroponic stigmas, which both exhibited ≥17 % crocin and >50 mg GAE g^{-1} phenolics, therefore project furthest along Dimension 1, corroborating earlier multivariate work where the best commercial saffrons also clustered on a crocin-phenolic pole (Irfan *et al.*, 2020).

Dimension 2, by contrast, separates samples whose antioxidant efficacy is driven by highly reactive carotenoids from those richer in flavour precursors. The sole strong positive arrow is DPPH activity, reflecting the well-documented potency of crocin and safranal in free-radical assays (Mohammadi *et al.,* 2023). Down-pointing loadings for picrocrocin and flavonoids indicate that tissues accumulating the bitter glycoside and flavonols tended, in this dataset, to show lower mass-specific DPPH values. This inverse relationship is not universal, flavonoids are typically antioxidative but it is consistent with treatment-specific trade-offs reported in controlled saffron experiments where cytokinin enhanced picrocrocin yet diluted crocin-derived antioxidant power (Singh *et al.*, 2023). Safranal, the principal aroma volatile produced from picrocrocin during drying (El Midaoui *et al.*, 2022), plots intermediate between the two quadrants, implying that aroma can be improved without large penalties in radical-scavenging capacity provided crocin remains abundant.

Mapping treatment scores (not shown) onto the same plane confirmed these biochemical syndromes. Kinetin-treated hydroponic samples and the Kishtwar field control co-located in the high- Dimension 1/low- Dimension 2 quadrant an optimum that marries intense pigmentation with balanced flavour whereas IAA-treated plants, despite modest crocin, plotted high on Dimension 2 due to exceptionally low DPPH IC_{50} values, suggesting induction of alternative antioxidative systems. GA_3 treatments, known to enlarge floral organs while moderating crocin density, occupied an intermediate position, and ABA

samples were extreme outliers at the origin of Dimension 1, confirming the hormone's suppressive effect on apocarotenoid synthesis.

Collectively, the loading pattern validates the use of PCA as a decision tool: elevating Dimension 1 should be the primary breeding or agronomic target because it simultaneously raises the three market-defining quality indices colour (crocin/crocetin), total phenolics and, indirectly, aroma while Dimension 2 can be fine-tuned by balancing antioxidant power against flavour intensity. The factor map thus provides a quantitative rationale for favouring cytokinin over ABA regimes in hydroponic production and for adopting cultivation conditions (e.g., the Kishtwar terroir) that naturally bias stigmas toward the high- Dimension 1 sector.

CHAPTER 6

SUMMARY AND CONCLUSION

The study aimed to assess the morpho physiological, floral, corm, biochemical, and antioxidant parameters of saffron in response to controlled environmental conditions and exogenous application of plant growth regulators (IAA, kinetin, GA_3, and ABA). Through meticulous experimentation and systematic analysis, the research sought to determine whether hydroponic cultivation, supplemented with specific hormones, could not only replicate but potentially surpass traditional field-based practices in terms of growth performance, flower yield, and quality attributes. The findings provide a comprehensive understanding of saffron's adaptive responses under varying cultivation systems and offer new insights into sustainable and precision-driven saffron production strategies.

6.1 Morphological parameters

Hydroponic saffron treated with kinetin (1 ppm) and GA_3 (50 ppm) achieved leaf counts (21.0 and 19.3 leaves/plant, respectively) and plant heights (16.0 cm for kinetin; 23.7 cm for GA_3) comparable to or slightly below soil-grown controls (22.2–23.0 leaves; 24.9–27.1 cm) . Leaf chlorophyll (SPAD) was highest under GA_3 (53.8) and ABA (49.6), intermediate with kinetin (36.8), and lowest in IAA (30.6) and the Kashmir control (30.8). Fresh leaf weight remained uniform (15–16 g/corm) across treatments, but dry leaf mass peaked with IAA (7.91 g) and kinetin (7.53 g), while ABA-treated leaves retained water (15.41 g fresh but only 2.16 g dry) .

6.2 Floral parameters

Flower weight per corm was maximized by GA_3 (404.6 mg) and soil controls (392–427 mg), intermediate with kinetin (315.1 mg), low with IAA (198.6 mg), and negligible under ABA (203.6 mg). Flower number mirrored this trend: kinetin (1.83 flowers/corm) and GA_3 (1.67) matched field norms (1.50–2.00), whereas IAA produced only one flower and ABA none. GA_3 also yielded the longest stigmas (1.71 cm vs. 1.22–1.38 cm in controls), while ABA again failed to develop stigmas. Total dry stigma yield (per 100

corms) was highest with kinetin (7.65 g) and GA_3 (6.83 g), on par with soil controls (6.63–6.67 g), markedly above IAA (2.27 g) and ABA (0 g).

6.3 Corm proliferation

IAA and kinetin dramatically enhanced daughter corm numbers (8.17 and 8.00 per mother, respectively) compared to GA_3 (4.33) and controls (6.83–7.83), while ABA suppressed proliferation (2.00). Average daughter corm weight was heaviest with kinetin (3.03 g), followed by IAA (2.55 g), GA_3 (2.07 g), and controls (1.55–2.38 g), with ABA again at the low end (0.58 g).

6.4 Biochemical composition

Kinetin-treated stigmas exhibited the highest apocarotenoid levels, with crocin reaching 17.40 % and crocetin 14.30 % of dry stigma weight—values statistically indistinguishable from the Kishtwar soil control (16.50 % and 13.80 %, respectively). GA_3 also promoted substantial pigment accumulation (15.20 % crocin; 13.10 % crocetin), whereas IAA-treated stigmas contained markedly lower levels (10.15 % crocin; 9.20 % crocetin). ABA treatment completely suppressed crocin and crocetin synthesis, resulting in 0 % for both compounds.

In terms of bitterness and aroma precursors, picrocrocin content peaked under kinetin (12.35 mg g^{-1} dry stigma) and soil controls (12.00 mg g^{-1}), was moderate with GA_3 (9.80 mg g^{-1}), low in IAA treatments (5.45 mg g^{-1}), and undetectable in ABA samples. Safranal followed a parallel trend, with kinetin yielding 4.25 mg g^{-1} and GA_3 3.60 mg g^{-1}, compared to 2.10 mg g^{-1} in IAA-treated stigmas and none in ABA.

Analyses of vegetative tissues revealed that ABA and kinetin stimulated the highest accumulation of total phenolics (ranging from 18.5 to 21.2 mg GAE g^{-1} FW in tepals and leaves) and total flavonoids (9.8–11.5 mg QE g^{-1} FW), indicative of stress- and cytokinin-induced secondary metabolism. IAA treatments produced the lowest phenolic and flavonoid levels (≈12.0 mg GAE g^{-1} FW and 6.5 mg QE g^{-1} FW), with GA_3 and soil controls intermediate. Proline content reflecting osmotic adjustment was highest under

ABA (2.8 µmol g^{-1} FW), moderate with kinetin (1.6 µmol g^{-1} FW) and GA_3 (1.4 µmol g^{-1} FW), and lowest in IAA and controls (0.8–1.0 µmol g^{-1} FW).

6.5 Antioxidant parameters

Antioxidant activity (DPPH IC_{50}) was strongest (lowest IC_{50}) in ABA-treated stigmas and in kinetin-treated tepals and leaves, reflecting elevated phenolics, flavonoids, and proline, whereas GA_3 and IAA showed moderate activity and soil controls were intermediate. PCA analysis confirmed that kinetin aligned with high pigmentation and balanced flavor, while ABA samples occupied a distinct high-antioxidant, low-pigment quadrant.

Conclusion

- Hydroponic treatments with kinetin and GA_3 restored vegetative growth leaf number, plant height, chlorophyll content, and leaf biomass to levels comparable with conventional soil cultivation.
- Flower development under GA_3 and kinetin matched or exceeded field norms, producing robust flower biomass, multiple flowers per corm, and fully developed stigmas, whereas IAA was less effective and ABA inhibited floral formation.
- Corm proliferation was markedly enhanced by IAA and kinetin, yielding more and heavier daughter corms than controls, while ABA treatments suppressed corm multiplication.
- Biochemical analysis showed that kinetin- and GA_3-treated stigmas achieved apocarotenoid levels on par with the best soil-grown samples, IAA had moderate effects, and ABA completely blocked pigment synthesis.
- Antioxidant profiling revealed that ABA-treated stigmas and kinetin-treated vegetative tissues exhibited the strongest radical-scavenging capacity reflecting elevated phenolics, flavonoids, and osmoprotectants while GA_3 and IAA treatments showed intermediate activity.

Therefore, this study demonstrates that carefully selected growth regulators can transform hydroponic saffron into a viable alternative to traditional soil cultivation, delivering comparable or even superior performance in vegetative vigor, flower production, and biochemical quality. By harnessing the stimulatory effects of kinetin and GA_3, producers can tailor secondary metabolite profiles to meet market demands for color, flavor, and antioxidant properties, all while overcoming the constraints of geographic terroir. Moving forward, integrating these treatments into scalable hydroponic systems promises not only to boost yield and quality but also to pave the way for year-round, controlled saffron production that aligns with sustainable and precision-agriculture goals.

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CERTIFICATE – IV

Certified that all the necessary corrections as suggested by the external examiner and the advisory committee have been duly incorporated in the thesis entitled **"Studies of Saffron (*Crocus sativus* L.) Cultivated under Soil and Soilless Growing Conditions"**, submitted by **Ms. Marvi Sharma,** Registration No. **J-20-D-50-BS.**

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