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Mitigation of Varied Light-Induced Pigment Contents by Indole-Acetic Acid in Germinating Wheat Seeds

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ABSTRACT

Quality is a major component of light that affects the plant pigments. The effect of blue, green, and red parts of the white light was studied independently to evaluate the role of individual light in germinating wheat as compared to white light as a control. Chlorophyll *a* was exhibited in response to green, red, and blue light in decreasing fashion (5.446, 4.226, and 3.636, respectively) as compared to the effect of white light, showing a stressful condition where normal contents were more compared to others (6.992). Similarly, chlorophyll *b* contents were also decreased in similar trends in response to green, red, and blue (4.320, 3.787, and 2.081, respectively) as compared to the effect of white light (5.666). Proline contents observed in response to varied light showed a little stressful effect in the case of blue light. All other conditions were similar to those of the control. Carotenoid and anthocyanin content exhibited different responses to varied light as compared to white light. Carotenoid showed more content (4.744) in red light, similar to the control, but anthocyanine exhibited more content in blue and green light (2.943, 5.356). When all the conditions were subjected to Indole-acetic acid, a dramatic increase in the pigment contents of all the types was observed, showing a mitigating effect of growth hormone. Presoaking the seed in Indole acetic acid before sowing may be recommended for the potential research activities in the future.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major staple food for more than one-third of the world population and is the main staple food of Asia. The total production area for major crops in Pakistan is approximately 8.9 million hectares, though recent data indicates a national average yield that remains a challenge, with significant variation across regions and crops (Pakistan Economic Survey, 2022-2023). The most critical external factors governing seed germination and early seedling establishment are water availability, optimal temperature, oxygen diffusion, and, for many species, light conditions (Finch-

Savage & Bassel, 2016). Successful germination requires a precise combination of these variables, which varies considerably among plant species; these requirements are often closely adapted to the specific ecological conditions of a plant's natural habitat (Baskin & Baskin, 2014).

Light functions as both an energy source and a developmental signal for plants, with each wavelength exerting distinct physiological effects. The visible spectrum comprises short (blue/violet) and long (red/orange) wavelengths, all of which are utilized in photosynthesis—the process by which plants convert

light energy into chemical energy. While green and yellow wavelengths are less effective for direct photosynthetic drives, the combination of all wavelengths in full-spectrum white light is essential for balanced growth and development (Kusuma et al., 2020). Plants perceive multiple aspects of their light environment, including its duration, intensity, direction, and spectral quality. They adapt to these conditions through dynamic changes in pigment composition and the reorganization of the photosynthetic apparatus, a sophisticated form of acclimation (Gao et al., 2022).

This light-mediated control over growth and development is termed photomorphogenesis. Seedling establishment is a highly light-responsive, photomorphogenic process. As sessile organisms, plants rely on complex photoreceptor systems to sense fluctuating light signals and optimize their growth accordingly. These systems—including phytochromes, cryptochromes, and phototropins—monitor light direction, quantity, and quality to regulate gene expression throughout the life cycle, from germination to flowering (Paik & Huq, 2019). Light not only provides energy but also regulates fundamental processes like chlorophyll biosynthesis, where biosynthetic intermediates themselves play key roles in guiding chloroplast development and biogenesis (Pogson et al., 2015).

Auxins (IAA) are fundamental compounds that coordinate key developmental processes, including bud formation, root initiation, and cell enlargement. They also stimulate the production of and interact synergistically with other hormones; for instance, in combination with cytokinins, auxins regulate the transition of stems to flowering and govern the growth of roots, fruits, and stems. Furthermore, following pollination, auxin activity in seeds is crucial for triggering the synthesis of proteins essential for fruit development (Osborne et al., 2005). Modern research continues to elucidate the complex signaling pathways of auxin, highlighting its role as a central integrator of environmental and developmental cues (Meng et al., 2022).

Gibberellins (GAs) are a class of plant hormones that regulate a diverse array of developmental processes, including stem elongation, seed germination, dormancy release, flowering, and senescence. Gibberellins promote seedless fruit development, cell elongation, and bud breaking. During photomorphogenesis, light and GA

often play antagonistic roles, fine-tuning growth responses. As tetracyclic diterpenes, gibberellins are essential for seed germination across numerous plant species (Sawada et al., 2008; Calvo et al., 2004). Contemporary studies have detailed the molecular mechanisms of GA signaling, particularly its interaction with light perception pathways to control developmental transitions (Davière & Achard, 2022).

This study investigates how different light spectra regulate seed germination and early development, and how plant hormones like IAA and GA modify these responses. Unraveling this light-hormone crosstalk is essential to understand fundamental plant adaptation and has direct applications for enhancing crop resilience and precision agriculture in controlled environments.

MATERIALS AND METHODS

To study the effect of differential light with hormonal mitigation during wheat seed germination, the seeds were obtained from the Crops Section, Department of Agriculture, Muzaffarabad. All studies were carried out in the laboratories of the Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. Procured seeds were cleaned from the dust by washing with distilled water. To avoid contamination, seeds were washed with 0.1% HgCl₂ solution and then rinsed with cold distilled water to make them free of surfactant. Half of them were soaked in 10⁻⁴M Indole acetic acid, half of them in 10⁻⁴M Gibberellic acid, and the other half in distilled water to use as a control.

Seeds were placed to grow in the Petri dishes lined with a double layer of filter paper according to the treatments. Petri dishes were placed in red, blue, green, and white light environment specially made for the purpose; an isolated light cabinet was designed and installed in the lab. Light period was 14 hours dark and 10 hours light. After the completion of the germinating stage, the seedling was harvested, and different parameters were determined. All the samples were subjected to the pigment analysis. For the determination of chlorophyll *a* and *b* few discs from the seedling were taken and ground with the help of a mortar and pestle, and the tissue was homogenized completely. This resulting extract was collected in test tubes. After homogenization, extracts were centrifuged using a centrifuge machine. After centrifugation, the resultant extracts were immediately assayed spectrophotometrically at the wavelengths of 663, 537

and 547 (Sim and Gamon 2002).

Proline was estimated by Bates *et al.*, (1973) method at the wavelength of 520nm. Sulphosuccinic acid solution was formed by taking 3g of sulphosuccinic acid and adding 100 mL of water. Acidic Ninhydrine was prepped by using 2.5 g Ninhydrine, 60ml glacial acetic acid, 30 ml distilled water, and 10ml orthophosphoric acid. One or two discs of leaves were ground with the help of a mortar and pestle in 10ml sulphosuccinate acid solution. Add 2ml acidic Ninhydrine and 2ml glacial acetic acid to the extract. Boil and cool, and after cooling, 6ml toluene was added, shaken thoroughly, and then poured into a separating funnel. Proline was extracted and then assayed spectrophotometrically at a wavelength of 520nm. All the data were statistically analyzed using MSTAT-C computer package. The significance of data was tested by using analysis of variance (ANOVA).

RESULTS

Effect of differential light on shoot weight

The shoot weight of seedlings was assessed under various light spectra and hormonal treatments. Under control (H₂O) conditions, white light yielded the highest shoot biomass. Exposure to monochromatic light resulted in reduced growth, with red, blue, and green light causing decreases of 5.87%, 3.62%, and 2.40%, respectively, relative to the white light control. Treatment with Indole-3-acetic acid (IAA) under all light conditions consistently inhibited shoot growth. The most pronounced reduction of 42.02% occurred under white light, with decreases of 15.44% and 12.96% under red and blue light, respectively. Gibberellic Acid (GA) application produced differential effects dependent on light quality. It induced a significant increase of 49.1% in shoot weight under blue light compared to the water-treated control under the same light. In contrast, GA treatment resulted in minor reductions under white (-1.54%) and red (-6.25%) light, and a more substantial decrease of 24.27% under green light.

Effect of differential light on pigment concentrations

The study examined the effects of different light qualities and plant growth regulators on pigment concentrations, revealing distinct patterns. White light consistently promoted the highest accumulation of chlorophyll a, with red and blue light causing significant decreases of 32.09% and 35.93%, respectively, under water

treatment. Indole-3-acetic acid (IAA) treatment under white light slightly increased chlorophyll a by 4.27%, while gibberellic acid (GA) caused a dramatic 210.7% increase under the same light compared to the water control. For chlorophyll b, white light again showed maximum levels, and GA treatment markedly enhanced its absorption, showing increases of 63.85% and 95.0% under white and red light, respectively. Carotenoid content followed a similar trend, with white light being most favorable. IAA generally increased carotenoids, but GA had the most profound enhancing effect, boosting levels by 98.34% under white light. Anthocyanin synthesis was also greatest under white light for water-treated samples. IAA had a mixed role, suppressing anthocyanin under white light but increasing it significantly under red and blue light, while GA increased it under white light but not under red. Overall, white light was the most effective spectrum for pigment accumulation, GA acted as a potent broad enhancer for chlorophylls and carotenoids, and IAA's effects were more variable and dependent on specific light conditions.

Effect of differential light on proline

The seeds placed under red, blue and green light showed an observable change in the proline contents. In red light proline contents up to 18.76% increase as compared to white light. Similarly in blue light 31.59% increase as compared to white and in green light 18.18% increase as compared to white light. In normal light in H₂O treated seedlings showed proline contents while same light in IAA treated seedling showed increases up to 37.68% as compared to control. Red and blue light in IAA treated seeds revealed increase as compared to control. Green light in H₂O treated sample showed increase while in IAA treated samples showed up to -0.68% decreases as compared to control. In GA and H₂O treated medium same light showed different proline contents. Green and red light in sample which soaked with GA showed minimum proline contents when relate with controlled samples. In H₂O treated samples green light revealed (1.677) proline content increase 15.73% when compared to control. Under the influence of white light samples which were treated with H₂O having value (1.296) while GA Soaking samples revealed value (1.499) increase 13.54% in comparison with control samples.

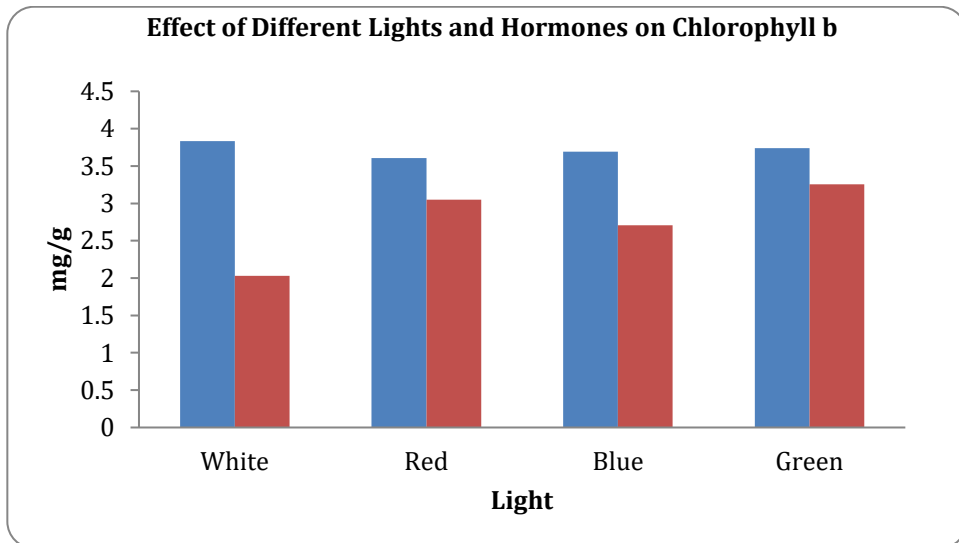
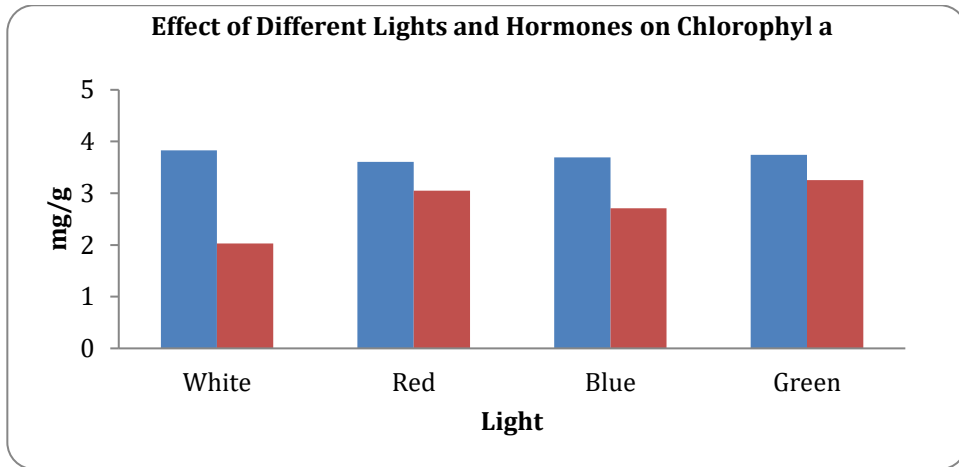
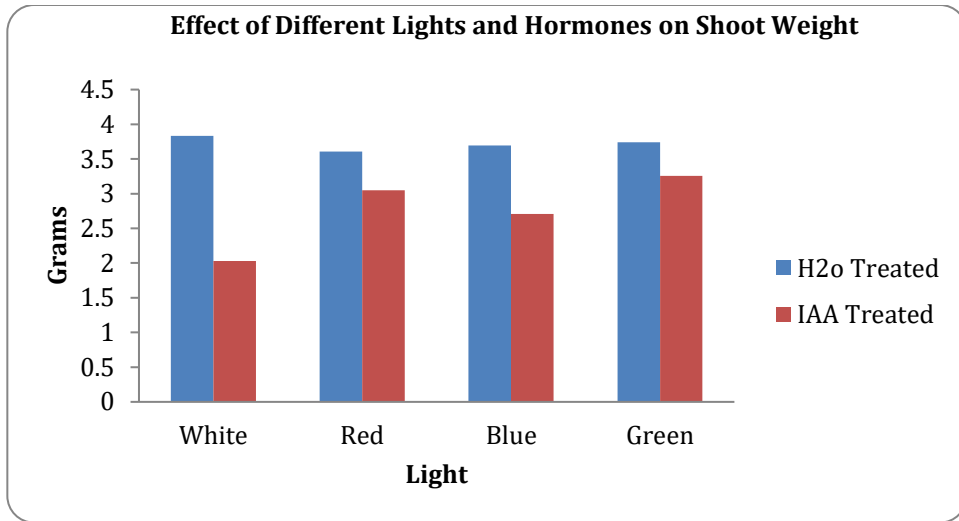


Figure 1 (a). Effect of different lights and hormones on plant growth and physiological parameters.

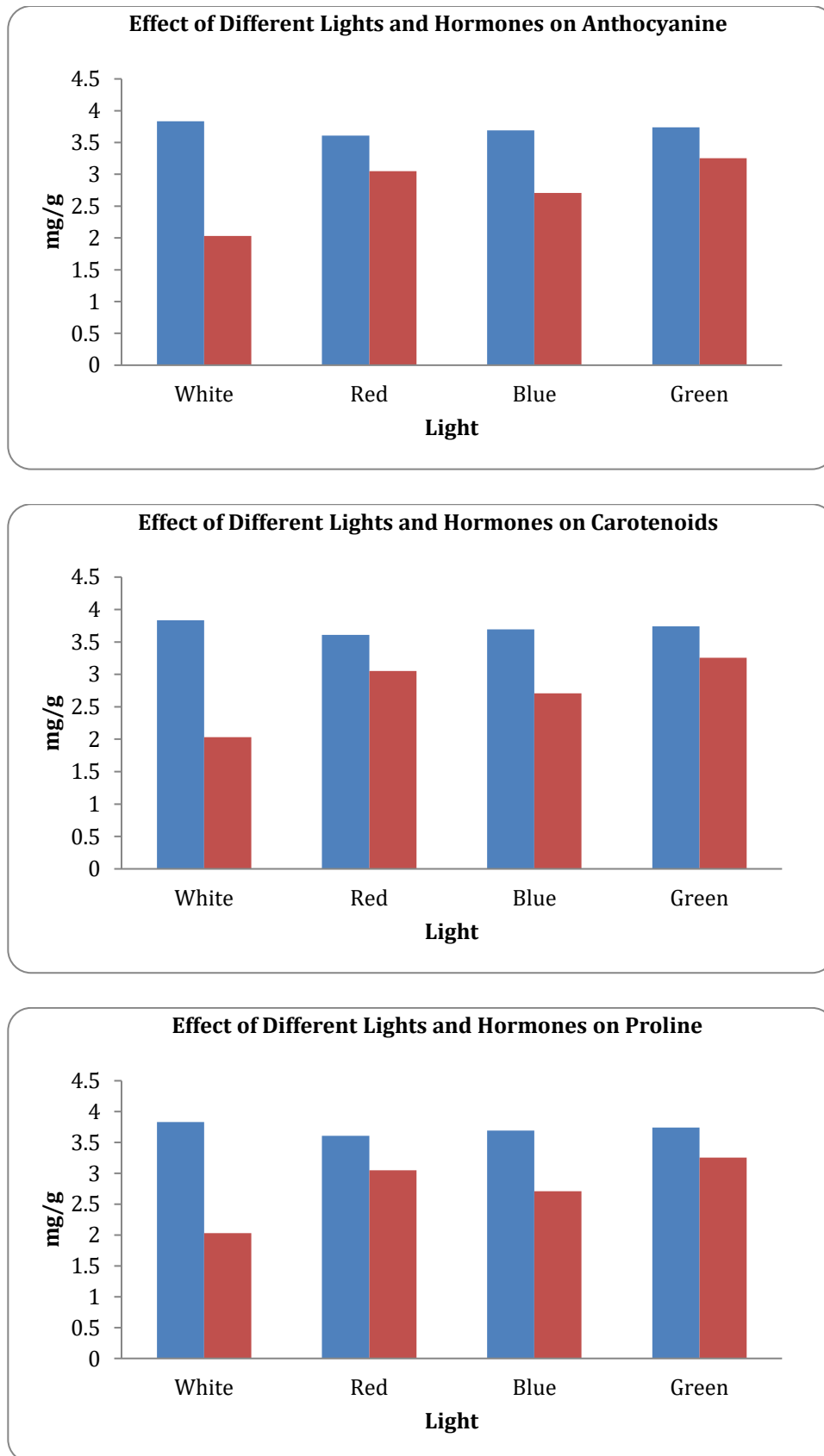


Figure 1 (b). Effect of different lights and hormones on plant growth and physiological parameters.

DISCUSSION

Light quality serves as a critical environmental signal, affecting plant growth and development through complex photomorphogenic responses. This study systematically evaluated the distinct roles of blue, green, and red spectral components, using full-spectrum white light as a control, in modulating shoot biomass and photosynthetic pigment accumulation in germinating wheat. Furthermore, the interaction between these light regimes and exogenous applications of the plant growth regulators indole-3-acetic acid (IAA) and gibberellic acid (GA) was elucidated.

Our findings confirm that white light is optimal for both shoot growth and the biosynthesis of chlorophylls, carotenoids, and anthocyanins. In contrast, monochromatic red, blue, and green light elicited stress-like responses, consistently reducing shoot weight and, in most cases, pigment concentrations compared to the white light control. This aligns with established literature where specific light wavelengths can limit photosynthetic efficiency and alter biomass partitioning (Kusuma et al., 2020). The application of IAA and GA revealed significant and differential hormonal modulation of light responses. IAA treatment generally exacerbated the growth-inhibitory effect of monochromatic lights, most severely under white light itself. This suggests that IAA's role in this context may involve sensitizing plants to light stress or altering resource allocation rather than mitigating it, consistent with findings that auxin signaling is integral to plant adaptation under variable light conditions (Müller-Moulé, 2020). Though its effect is highly dependent on the complex crosstalk between light signaling and hormonal pathways (Wang & Deng, 2023).

Conversely, GA exhibited a more complex, light-quality-dependent interaction. Its most striking effect was a profound, synergistic promotion of shoot growth specifically under blue light, where it increased biomass by approximately 49%. This finding highlights a key interaction where the hormonal signal (GA) and the environmental signal (blue light) converge to strongly promote elongation. This synergy likely involves shared signaling pathways, as both blue light photoreceptors (cryptochromes) and GA signaling are known to integrate to regulate processes like hypocotyl elongation (Wang & Deng, 2023). Under other lights, GA's effects were neutral or even negative, underscoring the

specificity of this interaction.

At the molecular level, these responses are mediated by photoreceptors such as phytochromes (responsive to red/far-red light) and cryptochromes (responsive to blue light). These photoreceptors perceive spectral cues and initiate signal transduction cascades that ultimately alter gene expression, affecting photomorphogenesis and pigment biosynthesis (Paik & Huq, 2019). The observed pigment profiles such as the promotion of anthocyanin by red light in certain treatments, consistent with Kim et al. (2004), and the varied effects on chlorophyll and carotenoid directly reflect the activation or repression of specific biosynthetic pathways by these light-hormone networks. The role of green light, often transmitted deeper into the canopy, appears significant in modulating pigment composition and biomass, potentially acting as a signal of shaded conditions, as suggested by its effects in this study and others (Smith et al., 2017).

In conclusion, this systematic analysis demonstrates that plant growth and pigment metabolism are not governed by light quality or hormones in isolation but are the result of intricate crosstalk. White light remains the most effective broad-spectrum signal for balanced development. The role of IAA appears contextually complex, while GA acts as a potent growth promoter whose efficacy is critically dependent on coincident blue light perception. These insights have practical implications for optimizing light regimes and pre-sowing seed treatments in controlled environment agriculture to enhance crop vigor and nutritional quality. Future research should focus on the downstream transcriptomic and proteomic changes underpinning these specific light-hormone interactions.

CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

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