The Effects of Light and Gibberellic Acid on the Growth of Wheat Plant (*Triticum aestivum* L.)

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Abstract

This study investigates the effects of exogenous gibberellins (GA) and the duration of light on the growth and development of wheat plants. To assess the impact of GA on seed germination and fungal disease, germination experiments are conducted. In the control group; 50% of seeds are sprouted: in which 50% of seeds are healthy and good while 50% of seeds are fungus-infected. The supplementation of 1% GA therapy is resulted in 46% normal growth and 53% fungal disease. While 3% GA remedy decreases fungal infection to 36% while boosting good germination up to 63%. While 5% GA solution produces the most remarkable outcome about 96% of seeds are sprouted normally and only 10% have fungal infection. In this study, several growth parameters are evaluated in response to light duration (full sunlight, 10 hours, 6 hours, 3 hours, and complete darkness) and GA treatment. Exposure to full sun without GA gives the longest root length of 12.15 cm; 3 hours of light produces the longest leaves (5.09 cm). Nevertheless, the most development under 3 hours of light exposure is found when GA is given—that is, longest roots (16.06 cm), intermediate shoot length (5.18 cm), and leaf length (7.24 cm), along with good fresh (0.47 g) and dry (0.18 g) masses. According to a fungal study, Fusarium oxysporum is the most typical pathogen; roots under full sunlight without treatment have infection rates of 100%, whereas those exposed to 6 hours of light have 66.66% infection rates. In contrast, untreated roots expose to full sun and 10 hours of light, Aspergillus niger has the lowest level of infection (33%). These results reveal that both light length and GA have major effects on several aspects of wheat plant growth, including germination rate, root and shoot growth, leaf length, and fresh and dry biomass.

Keywords: Gibberellic Acid, Abiotic Stress; *Triticum aestivum*; Endophytic Fungi; Plant Growth Regulators.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most consumable crops, that provide 20% of the calories and proteins to people worldwide

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(Venske et al., 2019). Wheat is one of the staple grains and an essential part of the diet for 1.2 billion people globally (Iqbal et al., 2018). Heat and deficiency pressures can expressly affect growing crops over short or extended periods, especially at delicate growth stages (Prasad et al., 2008). Dough wheat, along with maize and rice, is the primary food source for 35% of the world's population, with an annual report of 751 million tons (Hellemans et al., 2018). Wheat is considered an essential cereal crop around the globe and a major grain crop in Pakistan. Pakistan ranks 7th in wheat production with 26.4 million metric tons. In Pakistan, it is sown on around 9.17 million ha, which meets 83.5 % of food grain demand and the remaining 16.4 % is imported from other countries. Wheat yield varies from region to region in Pakistan due to the availability of water, use of fertilizers, varietal differences, farmer's access to technology, soil fertility and rainfall uncertainty (Arshad et al., 2023; Chandio., 2023). In India, 30.23 million hectares of wheat have reached full maturity, yielding 93.50 million tons (Tiwari et al., 2016). The production of wheat at the national level in 2016–17 is 2554 kilograms per hectare (Subedi et al., 2019). Researchers are concentrating their efforts on developing better agricultural techniques related to crop protection, harvesting, post-harvest technology, planting, nutrition, water management, and site preparation. The annual global crop, which covers 237 million hectares and yields 420 million tons (Isitor, 2007; Langer et al., 1991; Swetha & Sran, 2022).

The primary ecological region where more than 60% of wheat is produced is the Terai region. In Nepal, wheat production has increased constrained by the vast difference between the time grasp and the additional time; this difference might be closed by the availability of better seeds and a better set of techniques. There is reportedly a huge discrepancy between output potential and average national production (Timsina et al., 2019). The ultimate guarantor of global food security is thought to be cereal crops. Since the beginning of civilization, the golden ears of bread wheat have been seen as a symbol of world food security. With diminishing terrain and water resources and unpredictable meteorological circumstances, wheat output must be raised by 60% to reach 840 million tons by 2050 (Sharma et al., 2015). The main driver of the greenhouse effect and rising global temperatures is the generation of harmful gases like CO_2 (Raza et al., 2019). Due to its vulnerability to climate change and weather extremes such as temperature shifts, uneven rainfall patterns, and increase in frequency and severity of floods and droughts, agricultural zones are more affected than other zones. According to field research, between 1980 and 2015, heat and drought cause an up to 21% decrease in wheat yields. Worldwide, plants are frequently subjected to heat and drought conditions, which reduce crop yield. Heat and drought together have a greater impact on crop output than either condition does separately.

Between 17 °C to 23 °C is the ideal temperature necessary for the growth and development of wheat plants. Wheat seeds germinate between 3 and 4 °C at the lowest temperature, and flowering happens above 14 °C. Temperatures that are higher than the ideal range can significantly harm wheat crops (Porter & Gawith, 1999). Gibberellins (GAs) are a class of tetracyclic diterpenes well known for their impact on flower and fruit development, flower and trichome initiation, and leaf and stem expansion. (Yamaguchi, 2008). Gibberellic acid (GA) is a plant hormone that is produced in the roots of the plant and plays an essential role in plant growth and development. Numerous biological plant growth and development processes, including seed germination, leaf enlargement, stem elongation, and the development of reproductive organs, are influenced by bioactive gibberellins (Srivastava, 2002).

Gibberellins (GA) are essential plant regulators for a variety of developmental activities, such as seed germination, lengthening of stems and leaves, production of pollen, and induction of flowers. Since mutant plants lacking GA display a dwarf phenotype and delayed flowering as a result, giving GA to these plants restores their normal growth. Due to its favorable effects on plant growth and development, gibberellic acid (GA_3), a natural plant regulator, finds several uses in the agriculture and horticulture sectors. Several crops and ornamental plants have shown their beneficial impacts by displaying improved seed germination (Lee et al., 2016). Temperature and seed moisture content have an impact on germination. Seed priming is a widely used and effective approach for controlling the germination process. This technique is helpful to accelerate uniform emergence and increase plant vigor and growth (Siddik et al., 2015). Heat stress, which is defined as temperatures above the critical threshold, can vary between crop growth and developmental phases and impact several physiological processes, ultimately lowering grain output (Djanaguiraman et al., 2018).

Gibberellic acid is a substance that helps plants grow, develop, and respond to stressful environmental factors like drought (Patel et al., 2019). Allelic variation in the genes controlling growth habit (VRN) and photoperiod response favors wheat's ability to adapt to a variety of settings (PPD) (Kippes et al., 2020). Additionally, it changes the age of the leaves and enhances the source of physiological power by boosting chlorophyll content. By encouraging seed elongation development in cereal seeds, GA_3 hastens seed germination. One commonly used gibberellic acid (GA_3) lengthens stems, multiplies the number of flowers on each plant, and boosts output (Kurepin et al., 2014). Gibberellins (GAs), which contain tetracyclic and diterpenoid chemicals, are naturally occurring plant growth regulators. The proper location of bioactive GA in plants or tissues that are targeted by bioactive GAs to commence their activity has not yet been proven despite significant attempts to understand GA production and movements. A dwarf plant bioassay and its quantitative analysis identified the presence of GA in the shoot apices, young leaves, and flowers, which are all actively growing tissues.

In-depth research has been done on the photosynthetic parameters, phenotypes, stem mechanical characteristics, and yield dynamics of soybeans, while little research has been done on the important internodes that control the lodging of soybean plants in shade, their fiber composition, anatomical structure, and GA regulatory mechanism. The proposed study examines the stems' anatomical structure, fiber composition, GA_3 content, and the relative expression of the GA enzyme genes related to metabolism in the stems. Similar roles for GA are played in wheat in reducing the detrimental effects of cold on seed germination and seedling establishment. The goals of this study are to investigate the effects of exogenous Nitric oxide (NO) and GA on seed germination and seedling growth at low temperatures, as well as to demonstrate the underlying physiological mechanisms. The findings ought to be useful in examining realistic strategies to enhance seed germination and seedling establishment in wheat under low temperatures. To find out the level of gibberellic acid, the minimum and maximum amounts of gibberellic acid are used to determine the enhancement of the physical activity of plants. Light is important in terms of a source of energy and signaling activities in plants. That has a major impact on growth, development, and structural characteristics. Light intensity is closely related to morphological structure and material accumulation of plants, and both low and high light are detrimental to plant growth (Lv et al., 2021) When plants get exposed to low-light environments for long periods, they merely develop a series of shade-tolerant mechanisms to get adapt to such environments, such as increasing plant height in adaptive manner to obtain more light energy, by decreasing leaf area, energy consumption and transpiration respectively, promoting plant stem elongation, decreasing root biomass, and enhancing apical.

Methodology

Collection of Seeds Samples

A complete randomized design (CRD) is adapted for all experiments. In this study, the Gulzar-19 variety of wheat (Triticum aestivum) is collected from the Gullo dehri farm located in the area of Women University Swabi, Khyber Pakhtunkhwa, Pakistan.

Germination Test

A germination test is performed by using sterilized filter paper on sterilized Petri plates; a total of 24 Petri dishes are used in this study. Gibberellic acid is used as a seed dressing to determine the effect on germination, with different concentrations of 1%, 3%, and 5%, and non-treated seeds are considered as controls. Each treatment is used in triplicate. A total of ten seeds are placed on each Petri plate.

Preparation of Gibberellic Acid Solution

In the current study, three different concentrations (1mg/100ml, 3mg/100ml, 5mg/100ml) of gibberellic Acid are used. Light stress is also applied by providing the light at different periods (Control (normal daylight), 3 hours, 6 hours, and 10 hours' light duration, respectively.

Screen Experiment

The current study is conducted at the Laboratory of Botany, Women University Swabi, Khyber Pakhtunkhwa, Pakistan. Healthy wheat seeds are washed three times with autoclaved distilled water, followed by sterilization with ethanol (70%) and washing with distilled water three times. The sterilized seeds are soaked in different concentrations of GA_3 in petri dishes. Control seeds are treated with distilled water. The experiment is carried out at 27% humidity and 20-22°C with a photoperiod of 3,6, and 10 hours of light, respectively, with controls (fully daylight and fully dark conditions). After 7 days, the seeds fully germinate.

Field Experiment

Sterilized 24 plastic pots are used. Each pot is filled with 460.5g of sterilized soil and labeled. After providing the stress, simultaneously growth hormone gibberellic acid is added to the stress of light effect. The experiment is conducted at the greenhouse under fully controlled conditions at 22-25°C with a photoperiod of 3-hour light,6-hour light, and 10-hour light for long day exposure treatment and the control is fully daylight and another control for dark light. After 10 days the seeds started to grow.

Growth of Plant

After 45 days, plants are harvested and phenotypic data such as root length, shoots length, leaf length, and plant weight of wheat plant is recorded.

Isolation of Root-rotting Fungi

After data collection, Wheat roots are cut into 1 cm pieces. Each petri plates contain five pieces of 1cm wheat root. Roots of wheat plants are thoroughly dried on filter paper after sterilization for 1 minute to remove the surface sterilized 70% Alcohol and 30% water solution. Sterilized 24 Petri dishes, forceps, and all equipment are used in the process in an autoclave machine 24 petri plates are filled with PDA solution (Potato juice 15gm agar and 5g sugar solution) is added then the potato juice is poured into Petri dishes then put in laminar flow for one hour and then put the 24 petri plates in an incubator for fungus. The petri plates are incubated for 7 days. Fungi development on the root surface is identified through a microscope and find out their colonization and infection percentage by following the formulas. RPO (Relative Percent Occurrence) or infection incidence of fungus is calculated by dividing the number of samples having fungus by the total no of samples, multiplied by 100.

$$\text{Mean} = \frac{\text{Number of Petri plates}}{\text{Total number of plates}} \times 100$$

$$\text{Infection\%} = \frac{\text{Number of plates infected}}{\text{Total number of plates}} \times 100$$

$$\text{Colonization\%} = \frac{\text{Number of seeds infected}}{\text{Total number of seeds}} \times 100$$

Statistical Analysis

A complete randomized design is adapted for all experiments. The statistical analysis is performed using Graph Pad prisism9.0.0(121) software.

Result and Discussion

Seed Germination

Wheat seeds are treated with three different GA levels (1%, 3%, 5%) for 15 minutes to study the impact of gibberellic acid (GA) on seed germination. As shown in Table 1, the results revealed a strong effect of GA concentration on seed germination and fungal infection. In the control group, only 50% of the seeds are germinated successfully, with the remaining 50% exhibiting fungal infection. Slightly better results 46% of seeds germinated without infection and 53 % showed fungal infection resulting in 1% GA application. With 3% GA, where 63% of seeds are healthy and 36% fell prey to fungus, a more marked impact is seen. The 5% GA solution has the greatest improvement in producing a 96% normal germination rate and just 10% fungal infection.

These results provide strong evidence that GA therapy, especially at higher levels (3% and 5%), might notably increase seed germination rates while at once lowering wheat seed fungal infectivity rates as shown in Figure 1.

Table 1: Effect of gibberellic acid period (h) on seedling traits of wheat seeds.

Index	Germinated & Uninfected (%)	Un-germinated (%)	Infected Germinated (%)	Un-infected (%)
Without (GA) Control	50	0	50	0
GA 1%	46	0	53	0
GA 3%	63	0	36	0
GA 5%	96	0	10	0

A
B
C
D


Figure 1: Effect of Gibberellic acids on the germination of wheat seeds. (A) 5% GA, (B) 3%GA, (C) 1% GA, (D) Control.

Use of plant growth promoting gibberellic acid and light stress. To investigate the effect of light length and gibberellic acid (GA) a pot experiment is conducted. Different light regimes are applied to wheat plants ranging from full sunlight to complete darkness.

Phenotypic Effect of Gibberellic Acid and Light on Wheat Seeds

Figures 2 and 3 shows the effect of light duration and GA treatment on several growth features. In full daylight, plants raised without GA have the longest root length of 12.15 cm, while those exposed to 3 hours of light show the maximum leaf length (5.09 cm) as shown in Figure 4.

Plants grown under 3 hours of light show the most encouraged growth closest to GA; they have the longest roots (16.06 cm), moderate shoot length (5.18 cm), and significant leaf length (7.24 cm). Moreover, these plants are displayed fairly fresh weight (0.47 g) and dry weight (0.18 g) as shown in Figure 5.

Figure 2: Combine Effect of light and Gibberellic acids on the growth of wheat plant

Figure 3: Effect of dark/ light and gibberellic acids on the growth of wheat plant.

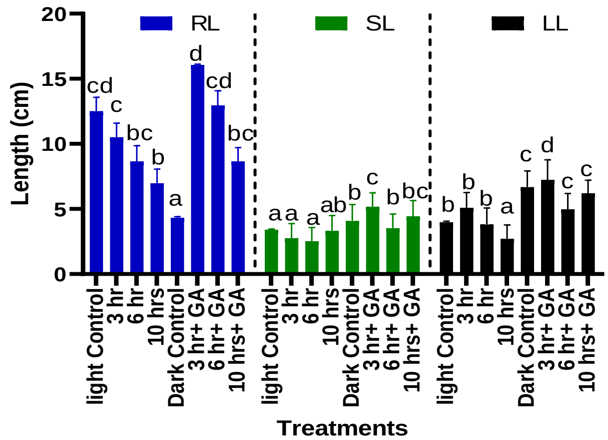

Figure 4: Effect of light on Root length (RL), Shoot length (SL), and Leaf length (LL) under normal and stress conditions supplemented with GA.

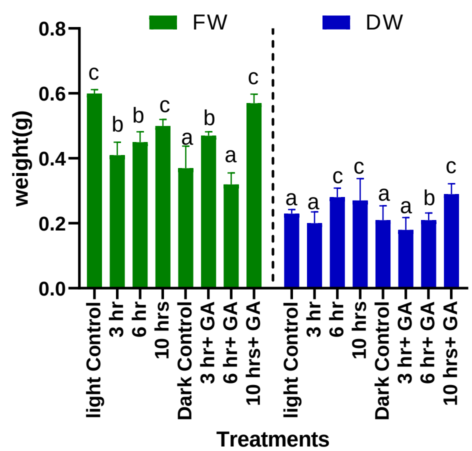

Figure 5: Effect of light on plant fresh and dry weight under normal and stress conditions supplemented with GA.

Infection Percentage of Fungus Associated with Roots

The dark region shows *Fusanium* in infected percentage (100%), is highest and in Control, while in 3 Hour series, it is lowest (66.6 percent). The Control, Three Hour+G.A., series displays the Rhizopus in infected percent of 66.6%. The Control, Dark, 3 Hour show the highest Alternaria in infected percent (66.6%) and 10 Hour+G.A shows the lowest Alternaria in infected (33.3%). Infected percentage by highest Aspergillus Niger is seen in The Control, 6Hour program (66.6%) and in Dark, 6Hour, 3hour + G.A shows the least *Aspergillus Niger* (33.3 percent). The infected percentage of highest *Fusanium Oxyponum* is seen in 10 Hour show (100 percent) and lowest in 6 Hour show (66.6%) and G.A, 3Hour show 6Hour+. The 3Hour+G.A indicates in infected proportion (33.3%) the *Aspergillus Flavus* as shown in Table 2.

Table 2: Effect of GA and light concentrations on wheat plants with the root pathogens.

S.No.	Treatment	Pathogens	Infection (%)	Colonization (%)
01	Control+full daylight effect	Fusanium oxysporum	100%	26.6%
		Rhizopus stolinefer	66.6%	26.6%
		Alternaria alternate	66.6%	13.3%
		Aspergillus niger	33.33%	13.3%
02	10-hour Light effect	Aspergillus Niger	33.33%	26.6%
03	6-hr Light effect	Fusanium Oxyponum	66.6%	26.6%
04	3-hr Light effects	Fusanium Oxyponum	66.6%	26.6%
		Alternaria alternate	66.6%	13.3%
05	Control in the Dark (without light effect + gibberellic acid)	Fusanium solani	100%	66.6%
		Alternaria alternate	66.6%	26.6%
		Aspergillus niger	33.3%	66.6%
06	10-hr Light effect + Gibberellic acid	Fusanium Oxyponum	66.6%	26.6%
		Alterneria alternate	33.3%	13.3%
07	6-hr Light effect + Gibberellic acid	Aspergillus Niger	33.3%	13.3%
		Fusanium Oxyponum	66.6%	13.3%
08	3-hr Light effect + Gibberellic acid	Aspergillus Flavus	33.3%	66.6%
		Macor	66.6%	13.3%
		Fusanium Oxyponum	66.6%	40%
		Aspergillus Niger	33.3%	13.3%

Colonization percentage of fungus associated with roots

The smallest *Fusanium* in the percentage of colonization (26.6%) comes from the 3 Hour; otherwise, the largest *Fusanium* in the percentage of colonization (66.6%) comes from the Control. The controls show the greatest Rhizopus in colonization percent (26.6%) as well as the least Rhizopus in colonization percent (13.3 percent). The darkest shows the lowest *Alternaria* colonization percentage (13.3 percent) as well as the highest *Alternaria* in colonization percentage (26.6%). *Aspergillus niger* in colonization rate is highest on the Dark show (66.6 percent) and middle on 6 Hour (26.6 percent). In colonization percent (13.3%), control, 6Hour +G. A, 3 Hours +gibberellin all exhibit *Aspergillus niger* the fewest. In terms of percentage of colonization, the 3 Hours +gibberellin have the highest *Fusanium oxyponum* (40%), and the medium *Fusarium oxyponum* (26.6%) is displayed elsewhere in 10 Hour, 10 H, 6 Hours, 6 Hours. 6Hours+G.A shows the least *Fusarium oxyponum* (13.3 %) in terms of percentage of colonization. The three-hour +G.A. shown in Table 2 illustrates the *Aspergillus flavus* colonizing percentage (6.66%).

Discussion

Wheat is one of the earliest crops that fulfill a significant portion of the global human diet's energy needs. Its consumption has been increased recently as a result of the availability of a variety of wheat. The quality, intensity, photoperiod, and day, night cycle are only a few of the lighting-related elements that have an impact on plant growth. This study's primary goal is to determine how light and gibberellic acid affect the growth of wheat plants. Many plants elongate as a result of gibberellic acid, and they all grow up to their normal height. When the plants are returned to full daylight, the amount of the rise in grain weight shows that yield might be constrained by the size of the sink (grain number in the present case). It indicates that the rate of grain development is not influenced by the number of stem-sugars. Gibberellin is an important group of diterpene plant hormones that control diverse aspects of the growth and development of plants from germination to flowering to seed formation. GA_3 increases the number of wheat plants able to reach the threshold of inductive generative development. However, the timing of flowering is not affected by GA_3 (Skalicky et al., 2020).

The current study indicates that the frequent use of gibberellic acid (GA_3) increases the stem's length and the leaf's size. The results of this research are supported by the findings of Dubert et al. (1993), reported that the Gibberellic acid has improved the percentage of wheat plants that may cross the inductive generative development threshold. The current study show that the process of seed germination is improved in the presence of light. These findings are consistent with the results of Schütz, Milberg, and Lamont (2002) who stated that the seed's size has an impact on how it responds to light during germination.

Numerous tiny seeds respond to light, which shows that light functions as a depth-sensing mechanism to prevent seeds from burying themselves too deeply in the soil and causing harmful germination. The results show that photoperiod treatment is only applied in the current experiment during the spike development phase. The plant either goes through the regular photoperiod of the winter growing season or approximately 12 hours. Photoperiod treatments are only applied during the spike growth phase, which is situated between the final spikelet stage (Ghiglione et al., 2008). The plants that's are subjected to either the natural 12.5-hour photoperiod of the growing season or the natural photoperiod followed by a 6-hour light extension provided by a combination of incandescent and fluorescent lamps. The analysis's findings indicate that the technique of pre-sowing treatment of wheat seeds with gibberellic acids in distilled water for 3 hours, 6 hours, and 10 hours have high effects on its germination. For percentage of germination and rate of germination, pre-sowing treatment of amaranth seeds with distilled water for 3 hours and PEG 6000 at -10 bars for 3 hours is more effective than other treatments in amaranth cultivars (Moosavi et al., 2009). In contrast to the present study, previous research demonstrated that chlorophyll content in wheat plants is not affected by gibberellic acid (GA3) treatment following application. In our current experiments, light treatments administered to dark-grown plants intensified the growth pattern changes typically observed in dark-grown plants receiving no light exposure. According to Pendleton and Weibel, 1965 that the yield of wheat grain decreased significantly when the crop did not receive full sunlight.

The study by Kabir et al. (2020) demonstrates that gibberellic acid treatment promotes root growth in wheat plants. Notably, the treatment leads to a significant increase in root length relative to shoot length when plants are exposed to 6 hours of light.

Conclusion

This study investigates the importance of light in plant growth and the role of gibberellic acid (GA), a plant hormone, in regulating key aspects of plant development, including germination, elongation growth, and flower development. Winter wheat could serve as a more effective winter cover crop if its seedlings grow more rapidly. Additionally, GA application has been found to enhance seed germination, seedling growth, and plant development, with studies demonstrating its ability to regulate cell elongation and cell division. The interaction between light and GA has also been investigated, with findings suggesting that GA can mitigate the effects of suboptimal light conditions on plant growth. By understanding the complex relationships between light, GA, and plant growth, researchers can develop strategies to improve wheat crop yields and productivity

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