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INDOLE ACETIC ACID AND GIBBERELLIC ACID ENHANCE PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE OF *JATROPHA CURCAS* L. UNDER WATERLOGGING AND DROUGHT STRESS

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Abstract

Jatropha curcas L. (*J. curcas*) is an emerging biodiesel plant attracting the interest of researcher around the globe. The current study was performed to enhance the growth of *J. curcas* under water logged and drought stress condition through application of phytohormones. We treated the plants with 100 mM and 250 mM Indole acetic acid (IAA) and Gibberellic acid (GA) exogenously. The study was performed in pots in summer and winter separately. The results showed that, all the plants died in the winter due to frost condition which showed that *J. curcas* was highly susceptible to low temperature and frost condition. Further experiment was carried out on the plants grown in summer. Both the hormones increased the plant height under both the stresses while the length of root was considerably increased by GA in the water logged and drought stress conditions compared to IAA. The root and shoot fresh and dry weight were enhanced significantly by GA 250 mM.. Leaves and branches number, and stem diameter were greatly affected by water logging and drought stress, however both the hormonal application increased these parameters. Further the results showed that, GA 250 mM highly increased the proximate compositions in both the stresses followed by IAA 250 mM. These results collectively demonstrate that the external administration of IAA and GA stimulates the growth and development of *J. curcas* under conditions of waterlogging and drought stress by modulating various morphological, physiological, and biochemical parameters.

Key words: Drought, Gibberellic acid, Indole acetic acid, *Jatropha curcas*, Waterlogging.

Introduction

Jatropha curcas L. is a deciduous shrub of the family Euphorbiaceae grows in tropical and subtropical regions across the continents of America, Africa, and Asia (Gimeno *et al.*, 2012). This stem-succulent plant is tolerant to drought and salinity, contains high oils content, growing rapidly and highly capable of thriving in a broad range of agro-climatic conditions (Maes *et al.*, 2009; Divakara *et al.*, 2010; Silva *et al.*, 2010). *J. curcas* require minimal water and can grow in poor soil (Valdes-Rodriguez *et al.*, 2011). From a sustainability perspective, this plant produce extra opportunities for income, sustainable energy production on rural base, and protection and restoration of marginal soil (Pérez-Vázquez *et al.*, 2013). *J. curcas* has the ability to thrive in regions characterized by extreme climate and soil conditions, making it suitable for areas where many other agriculturally significant plant species cannot establish. (Francis *et al.*, 2005). *J. curcas* received attention due to its high oil contents in seeds which can be transformed to biodiesel, and because it can prevent soil-erosion under flooded conditions (Openshaw, 2000; Ogunwole *et al.*, 2008). It produces high quality oil and contain high number of octane compared to other oils, which make it a suitable alternative fuel. Its cultivation as a potential source of bio-diesel, is recommended by some governments and NGOs, on poor soil and marginal land (Verma *et al.*, 2012).

As *J. curcas* is fast growing even in minimal water condition, however optimal amount of water is crucial to given at a right time (Pérez-Vázquez *et al.*, 2013). The influence of water stress on growth of most of the commercial plants are well known. Generally, drought stress is recognized as a key plant growth limiting factor. For instance, drought effect photosynthesis, transpiration, chlorophyll and dry matter and restrict growth and development (Sarker *et al.*, 2005). However, there is very limited data available in *J. curcas* related to this issue. Indeed, no scientific information about quantitative demand of water and water use efficiency in *J. curcasz* are available at present (Achten *et al.*, 2008). However, it is known that *J. curcas* can grow well in limited water condition compared to other plants (Pérez-Vázquez *et al.*, 2013). It is studied previously that, water application increases seed and oil yields (Abou Kheira & Atta, 2009), while on the contrary, it is found that some plants affect oil yields by the amount of available water (Said-Al *et al.*, 2009). Depending on plant species, water effect the oil contents and other proximate compositions. Khalid (2006) reported that high soil water (125%) increases oil contents and carbohydrates while affect protein level in Ocimum species (Khalid, 2006). It is predicted that, it grows in almost all type of soil such as gravelly, sandy, saline, poorest stony soil, and even in the crevices of rocks (Al-Busaidi *et al.*, 2012). The plantation of *J. curcas* on degraded soil enhances soil fertility but the claim that it grows sustainably under drought stress has no solid scientific ground.

On the other hand, soil flooding is another serious environmental stress which is defined as when soil micro and macro pores are filled with water (Verma *et al.*, 2012). Filling the pores of soil affect aeration very seriously and eventually affect the plant growth performance negatively (Arbona *et al.*, 2008). In 1982, it was speculated that, water logging affect about 16% of the total world crop producing agro-climatic areas (Boyer, 1982). Flooding condition limiting the rate of gas exchange in water which reduce oxygen supply and affect the aerobic respiration of root and inhibit other bio-geochemical process in the soil (Visser & Voesenek, 2005). It is reported that water logging induces ethylene stress which results into reduced chlorophyll content, aging induction, leaf abscission, inhibits root elongation, and reduced growth and development (Ali *et al.*, 2018; Jung *et al.*, 2018). Root is an important sensory organ of plant that detect stress condition in the soil while the limiting oxygen in the rhizosphere significantly affect the development of root system (Jackson *et al.*, 1996; Drew, 1997). Water logging causes several serious problems such as reduced soil temperature, reduced microbial activities, reduced degradation of organic materials, and limiting release of nutrients from organic matter, limiting CO_2 accumulation, reduced availability of nutrients, and accumulation of toxic substance in the rhizosphere (Verma *et al.*, 2012). Study shows that, water logging decreased shoot and root length, soluble sugar, starch, and relative water contents in leaf of *J. curcas* (Gimeno *et al.*, 2012). It was further concluded that strong reduction in *J. curcas* growth, leaf area, leaf number, stem diameter, leaf weight, and stomatal conductance under water logging reduced biomass yield (Verma *et al.*, 2014). Reports indicate that waterlogging diminishes shoot growth by causing a decrease in leaf area expansion and triggering premature leaf senescence and shedding (Kozlowski, 1997; Mielke *et al.*, 2003). The detrimental consequences of waterlogging include the inhibition of leaf growth, decreased shoot and root growth, reduced overall plant biomass, and the acceleration of plant senescence leading to eventual mortality (Pociecha *et al.*, 2008).

Abiotic stresses severely affect plant growth and development which resulting in yield losses. Plants have evolved various defense strategies to cope these stress conditions. Hormonal signaling is an important defense mechanism for mediating plant responses to stress tolerance. In the past few years, many hormones related to biotic and abiotic stress tolerance have been characterized and have shown significant roles in plant responses to stress tolerance. Gibberellic acid (GA) is included one of the key plant growth regulator along with auxins, cytokinins, abscisic acid, and ethylene (Kende & Zeevaart, 1997). Basically, GA have been associated with alteration of plant stature and seed dormancy, however it has direct or indirect effects on the regulation of various traits of plant (Groot & Karssen, 1987; Wang *et al.*, 2017; Asif *et al.*, 2022). Researchers have reported that, GA enhance fruit yield and growth parameters in *J. curcas* (Ghosh *et al.*, 2010; Pan & Xu, 2011; Pan *et al.*, 2016). Recently, it is studied that the application of GA exogenously enhances the flower number and flower diameter in *J. curcas* resulting into increase yield (Hui *et al.*, 2016). There is lack of scientific data which evaluates the effect of application of GA exogenously on *J. curcas* agronomic traits under waterlogging condition. However, researchers reported that exogenous application of GA to *Vigna radiate* L. during waterlogging condition significantly promoted shoot length, shoot dry weight, root dry weight and stem diameter (Islam *et al.*, 2022). Additionally, indole acetic acid (IAA) can also regulate the plant tolerance to stress condition and modulate several processes during plant growth and development (Qin *et al.*, 2011; Du *et al.*, 2012). IAA is involved in the adventitious root development under waterlogging, which is a common adaptive responsive (Cisse *et al.*, 2022). IAA plays a crucial role in regulating cellular division, elongation and differentiation, which promote plant growth and development (Anfang & Shani, 2021). Previous studies also indicated that, exogenous application of IAA increased drought stress tolerance in *Trifolium repens* via regulation of ABA and JA related genes (Zhang *et al.*, 2020). Furthermore, exogenous IAA increased shoot fresh weight, stem fresh weight, fresh and dry weight of root in *Syzygium jambos* under waterlogging (Cisse *et al.*, 2022). Previously, we described that exogenous GA and IAA enhanced root shoot length, leaf and branches number and proximate composition of *J. curcas* (Jan *et al.*, 2022). It is also reported recently that, growth promoting bacteria also produces hormones such as, IAA and GA which significantly regulate plant growth and development (Moon & Ali, 2022). Nevertheless, there is limited knowledge regarding the physiological, morphological, and biochemical changes caused by the application of GA and IAA, as well as the ability of *J. curcas* to adapt to drought and waterlogging conditions. The knowledge gap restrict our capacity to evaluate and predict properly the agronomic performance of *J. curcas* under drought and water logging stress and exogenous application of GA and IAA.

Globally, the supply of fossil fuels is depleting, which is driving up costs and significantly straining developing nations' economies. Researchers are looking for sustainable and alternative fuel sources. *J. curcas* is one of the biodiesel plants that can be used as a source, and it is advised to grow it on marginal and waste land. In order to assess *J. curcas's* succession in the Kohat district, Pakistan we previously worked with the Directorate of Science and Technology (DOST) on a trial-based initiative. In Khyber Pakhtunkhwa, Pakistan, Kohat is a humid subtropical area with summer temperatures of 38ºC and winter temperatures of 7.4ºC. The yearly rainfall in this region is 52 mm in July and August, and the annual humidity is 43 and 42% in February and August, respectively (Fig. 1). However, our project's outcome was unsatisfactory. It is essential to look for potential *J. curcas* succession paths in the chosen area before pursuing large-scale plantations of the species. Thus, the current study's goal was to investigate how the *J. curcas* plant responded physiologically, morphologically, and biochemically to waterlogging and drought, as well as how exogenous GA and IAA hormone treatment helped to mitigate both stresses.

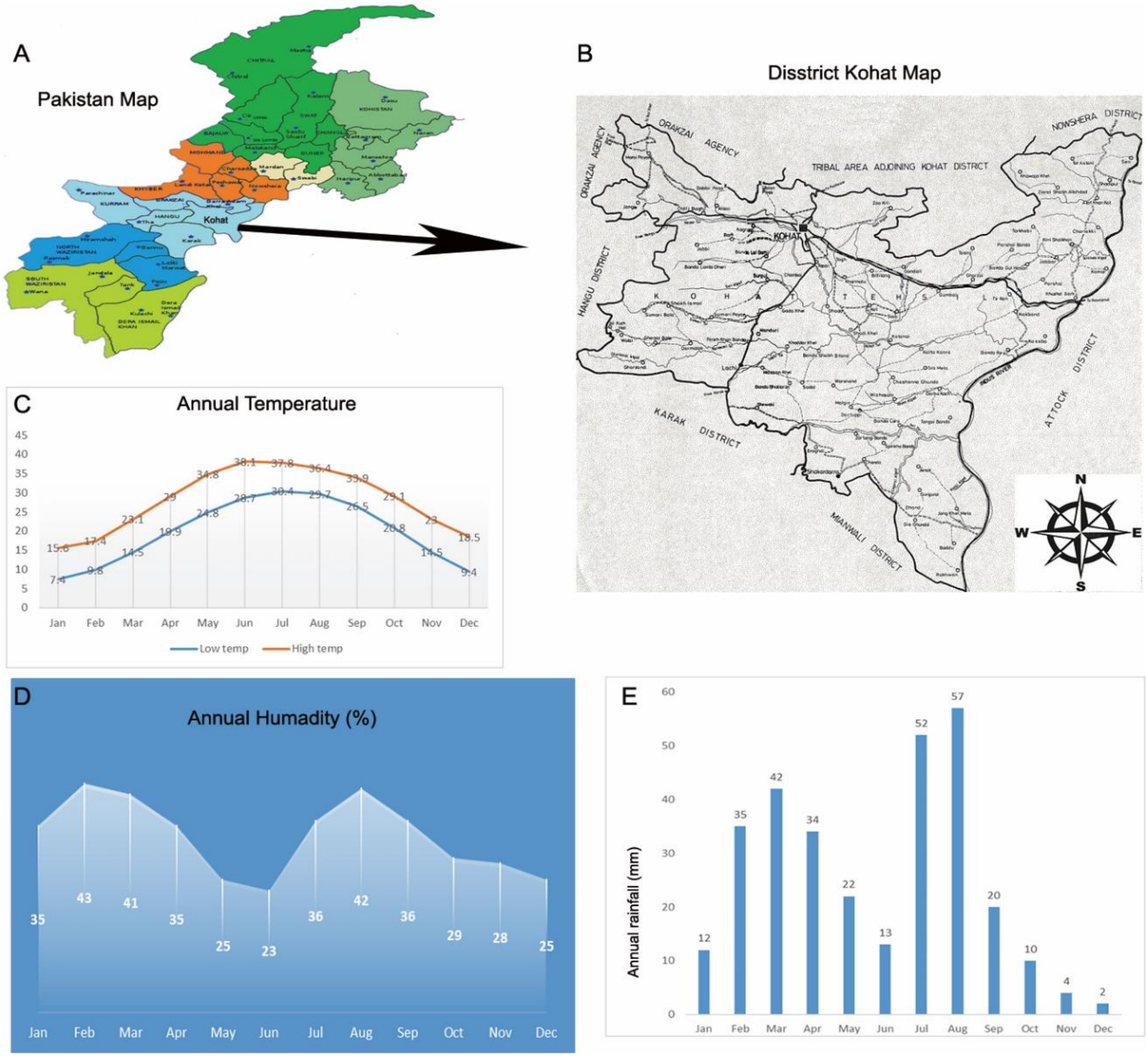


Fig. 1. Map and annual climatic condition of district Kohat. (**A**) Shows map of Pakistan, (**B**) shows map of district Kohat, (**C**) represent annual temperature of district Kohat on monthly base, (**D**) represent annual humidity of district Kohat on monthly base, and (**E**) represent the annual rainfall of district Kohat on monthly base.

Material and Methods

Experimental design and material used: The current study was performed in pots and used the local field soil. The equal size pots (23x17 cm) were filled with 2.5 kg soil equally. The plants were supplied by a standard distributor from Multan (Pakistan). Identical-sized single seedlings were cultivated in individual pots containing equal amounts of soil. Indole Acetic Acid (IAA) and Gibbrellic Acid (GA, GA_3) were exogenously applied as growth regulators under drought and water logged condition. The whole experiment was divided into 11 treatments such as control (Cont), waterlogged (WL), waterlogged+100 mM IAA (WL+ IAA100), waterlogged+250 mM IAA (WL+IAA 250), waterlogged+100 mM GA (WL+GA 100), waterlogged+250 mM GA (WL+GA 250), drought stress (DS), drought+100 mM IAA (DS+IAA 100), drought+250 mM IAA (DS+IAA 250), drought+100 mM GA (DS+GA 100), and drought+250 mM GA (DS+GA 250). Each treatment was replicated three times. All the plants were grown in natural environment for about four months from May to August. Control plants received weekly watering with one liter of water, while drought-stressed plants were watered every two weeks with half a liter of water. Waterlogged plants were continuously submerged in water by daily watering to maintain the water level at 3 inches above the soil surface. Number of leaves, branches number, and stem diameter were measured after each week while the root length, root and shoot fresh and dry weight and proximate composition analysis data was collected after the experiment was finished.

Parameters studied: To explore the impact of drought and waterlogging, as well as the alleviating effects of exogenous GA and IAA application on *J. curcas*, we conducted a comprehensive analysis of physiological, morphological, and biochemical factors. Regarding physiological parameters, we assessed root and shoot length, as well as the fresh and dry weight of both roots and stems. For morphological parameters, the leaves number, branches number, and diameter of stem were recorded. In terms of biochemical parameters, carbohydrate, protein, lipid, moisture, and ash contents were analyzed. Data collection occurred on a weekly basis over a four-month period.

Proximate composition analysis: To assess the impact of waterlogging, drought stress, and hormone treatments on leaf moisture content, fresh leaves were gathered randomly in triplicate from each group of treatment. The gathered leaves were rinsed with tap water, weighed, and noted as the fresh weight (Fw). Subsequently, the collected leaves were subjected to a 3-hour period at 105°C in an oven. After complete drying, the leaves were weighed again and noted as the dried weight (Dw). The moisture content was then measured using the following formula:

$$\text{Moisture content (\%)} = (\text{Fw-Dw}) \times 100$$

To investigate the percentage of ash content, we initially took 1 gram of leaves and thoroughly rinsed them to remove any impurities. Subsequently, the washed leaves were allowed to dry. These dried leaves were then subjected to heating in a muffle furnace with the temperature ranging 450°C to 550°C to eliminate moisture and other volatile constituents. Prior to and after the burning process in the muffle furnace, the leaf samples were weighed. The ash content was computed using the following formula:

$$\text{Ash (\%)} = (M_{ash}/M_{dry}) \times 100$$

where M_{ash} is the mass of fresh ash and M_{dry} is the mass of dry ash.

To evaluate fat content, we followed established by Aurea M. Almazan and Samuel O. Adeyeye (1998) (Almazan & Adeyeye, 1998). Approximately 1 gram of fresh *J. curcas* leaves were collected and then dried in an oven at 60°C for a period of 15 hours. Subsequently, the dried leaves were finely ground into powder. Then the powder was mixed with hexane and the fat was extracted by following the method of Soxhlet (AOAC, 1990). The extracted fat was further processed by treating it with methanolic sodium and methanolic boron trifluoride to transform fatty acid into methyl ester, following the method of Paquot & Hautfenne (1987). The consequential layer of hexane comprising the fatty acid methyl ester was dried at 90°C with nitrogen gas passing on its surface. The solution of hexane and methyl ester was then filtered using a 45 µm filter and subsequently injected (1 µl) into Shimadzu gas chromatograph equipped with a Perkin-Elmer PE-WAX capillary column (30 m × 025 mm) and a flame ionization detector. The 80°C temperature of column was increased to 260°C, held for 7 minutes, with a gas flow rate (H_2) of 30 cm/s. The fat percentage was determined on the basis of total area of fatty acids. All these evaluations were performed according to the method outlined by the AOAC (AOAC, 1990).

To evaluate crude protein, Micro Kjeldahl method was employed following the guidelines of AOAC international (Latimer, 2016). One gram of leaf samples was collected, finely powdered, and subjected to digestion with 15 ml of H_2SO_4 while heating, in the presence of K_2SO_4 and selenium, utilizing a heating block set at 420°C for a duration of 2 hours. Following digestion, the sample was neutralized by introducing NaOH, a step used to convert ammonium sulfate into ammonia. The ammonia was then distilled off in a flask, and boric acid was added to form ammonium borate. The remaining boric acid was titrated with H_2SO_4, using an endpoint indicator to investigate the net nitrogen content. The net nitrogen content in the residue was then multiplied by the conventional conversion factor of 6.25 and a specific conversion factor (Mæhre *et al.*, 2018). Carbohydrate content was evaluated via measuring the variance between the sum of all the proximate composition components from 100%, as described by (Marcel & Bievenu, 2012).

Soil analysis: For soil nutrients calculation, soil was characterized in the Barani Agriculture Research Center (BARS), Kohat. Two sites of the field were selected for soil sample analysis. The BARS report of soil is presented in (Table 1) already reported in our previous study (Jan *et al.*, 2022).

Statistical analysis

The experiments were carried out in triplicate, and the data from each replicate was combined. Data analysis was conducted using one-way and two-way ANOVA with Bonferroni post hoc test (* shows $p<0.05$, ** shows $p<0.01$, and *** shows $p<0.001$ significant difference). To evaluate the mean values of different treatments, a completely randomized design was employed. DATA were visually represented in graphs, and the statistical analyses were conducted via GraphPad Prism software (version 5.01, GraphPad, San Diego, CA, USA).

Results

***J. curcas* is susceptible to low temperature and frost condition:** The current study was carried out at the Kohat University of Science and Technology in Kohat, Khyber Pakhtunkhwa, Pakistan, in a natural setting. Initially, we conducted the experiment in winter but due to the frost in December, the young seedling could not survive as shown in (Fig. 3B). After plant growth failure in winter, the experiment was conducted in summer and continued until the end. Mature *J. curcas* plants in the field were also susceptible to frost and the flowers and the fruit were adversely affected during winter season (Fig. 3D, F, H). The young plants were also susceptible to the cool weather in the field while in the hot summer it grows well (Fig. 2). On the other hand, *J. curcas* seedlings, mature plants, flowers and fruits did well in the hot summer season (Fig. 3A, C, F, H). From these results concluded that, *J. curcas* was highly resistant to high temperature and susceptible to low temperature and could not survive in frost condition.

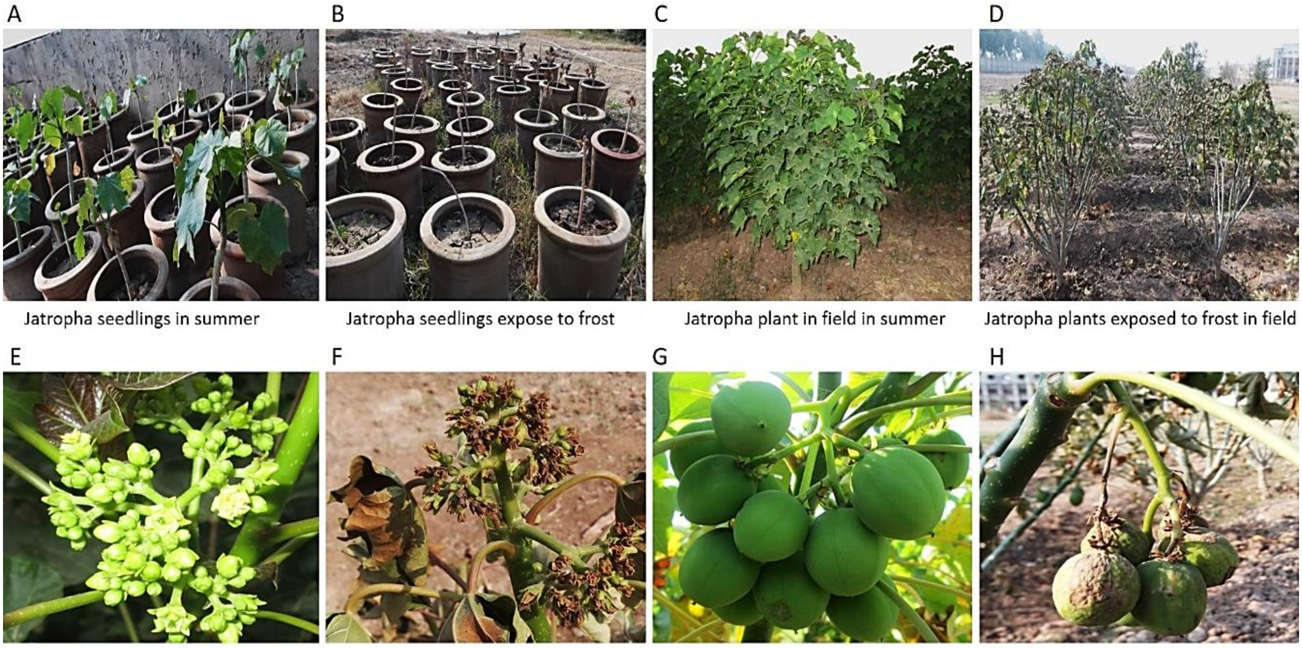
Table 1. Soil characterization.

Sample No.	pH	Electric conductivity ds/m	$CaCO_3$ %	Organic manure %	Nitrogen %	Texture
1.	7.51	0.55	14	0.69	0.0345	Clay loam
2.	7.31	0.67	16	0.759	0.0379	Clay loam

J. curcas in summer in field

J. curcas in winter in field

Fig. 2. Young *J. curcas* is susceptible to low temperature. Right picture shows one year old plant in the field in summer, while left picture shows one year young plant in the field in winter season.



Jatropha seedlings in summer

Jatropha seedlings expose to frost

Jatropha plant in field in summer

Jatropha plants exposed to frost in field

Jatropha flowers in summer

Jatropha flowers expose to frost

Jatropha fruits in summer

Jatropha fruits exposed to frost

Fig. 3. *J. curcas* growth and development pattern in winter and summer in Kohat region. (**A**) represent six month old seedlings in pots in the summer, (**B**) represent six month old seedlings in pots in winter under frost stress, (**C**) shows mature plant in the field in summer, (**D**) shows mature plants in the field in winter under frost stress, (**E**) shows flowers in summer, (**F**) shows flowers in winter, (**G**) represent fruits in the field in summer, and (**H**) represent fruits in the field under frost stress.

IAA and GA enhances *J. curcas* growth vigor under waterlogging and drought stress: In this study, we found that water logging and drought stress inhibited the growth parameters of *J. curcas* (Fig. 4). The waterlogging and drought stress significantly affected shoot length, shoot fresh and dry weight and root length (Fig. 4A, C, E). Shoot length of waterlogged plants was decreased 7% and drought stressed plant shoot length was reduced 4% after 17 weeks compared with control plants (Fig. 4A). Whereas, IAA and GA application significantly enhanced the length of shoot after 9 and 17 weeks compared with waterlogged and drought stressed plants respectively. Between the IAA and GA, GA 250 mM increased the shoot length 11% and 41% after 17 weeks under waterlogging and drought stress respectively. Waterlogging reduced the root length (53%) significantly, while drought stress showed non-significant increase compared with control plants (Fig. 4B). However, GA 100 mM and GA 250 mM significantly increased the root length under both the stresses while IAA showed non-significant result. GA 250 mM increased root length 158% and 100% in waterlogging and drought stress compared with waterlogged and drought stressed plants respectively (Fig. 4B). Waterlogging and drought stress significantly reduced shoot fresh weight (39, 47% respectively) and dry weight (28, 42% respectively), while root fresh weight was only reduced in waterlogging (27%) compared with control plants (Fig. 4C, D, E). Although, IAA promoted *J. curcas* shoot length under both the stresses but its effect on shoot fresh and dry and root fresh and dry weight was not satisfactory. On the other hand, both GA concentrations markedly increased the fresh and dry weight of the root and shoot; however, GA 250 mM produced better results than GA 100 mM. For instance, GA 250 mM increased shoot fresh weight 167%, shoot dry weight 108%, root fresh weight 201% and root dry weight 214% under waterlogging compared with waterlogging plants. Under the drought stress, GA 250 mM increased shoot fresh weight 231%, shoot dry weight 240%, root fresh weight 136% and root dry weight 153% compared with drought stressed plants. These results indicates that GA efficiently promote the growth of *J. curcas* plant under both the waterlogging and drought stress.

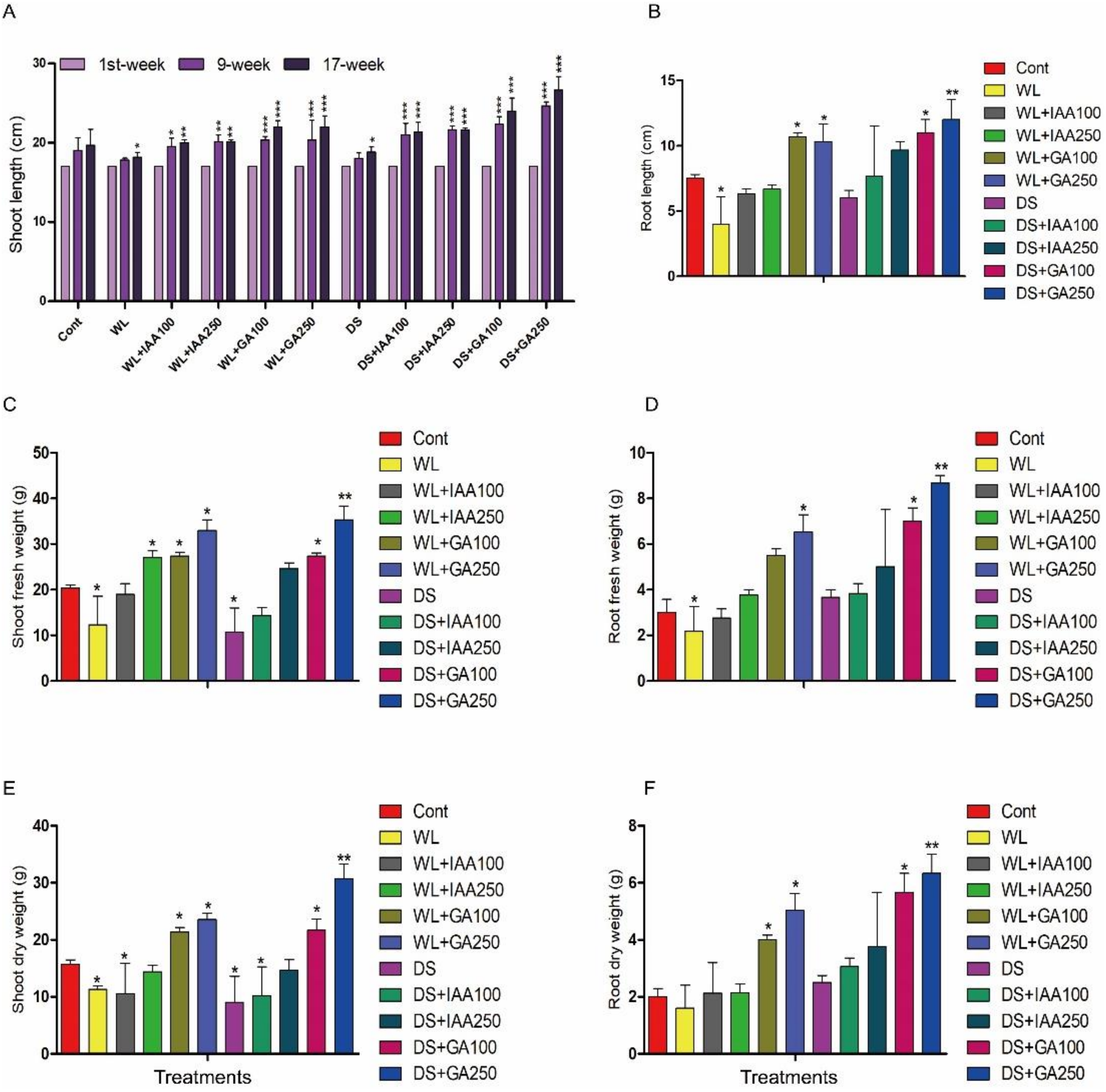


Fig. 4. IAA and GA effects on *J. curcas* root shoot length and fresh and dry weight. (**A**) Shows shoot length after three time points of 1st week, 9th week and 17th week, (**B**) shows root length, (**C**) shows shoot fresh weight, (**D**) shows root fresh weight, (**E**) shows shoot dry weight, and (**F**) shows root dry weight. Graphs show mean ± standard deviation, and asterisks show significant differences (* $p\leq0.05$, ** $p\leq0.01$, and *** $p\leq0.001$) according to two-way ANOVA and one-way ANOVA with Bonferroni post hoc tests. Waterlogged and drought stresses were compared with control plants while hormone applied plants under stress were compared with their respective stressed plants.

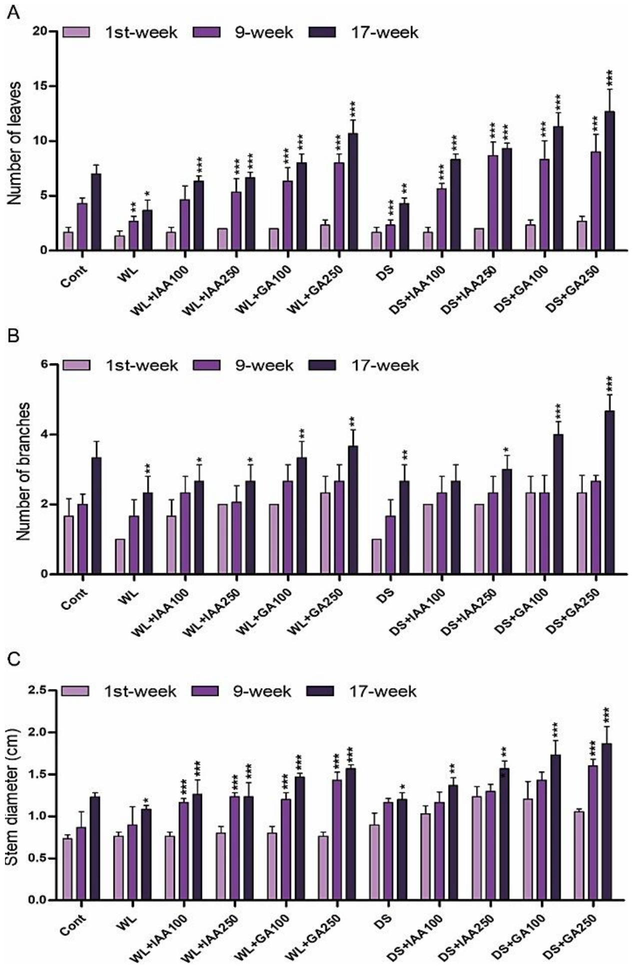


Fig. 5. IAA and GA enhance leaves number, branches number and stem diameter of *J. curcas* under waterlogging and drought stress. (**A**) Shows leaves number (**B**) shows branches number, and (**C**) shows stem diameter. The date were collected after three time points of 1st week, 9th week and 17th week. Graphs show mean ± standard deviation, and asterisks show significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$) according to two-way ANOVA with Bonferroni post hoc tests. Waterlogged and drought stresses were compared with control plants while hormone applied plants under stress were compared with their respective stressed plants.

Exogenous IAA and GA promote *J. curcas* morphology under stress condition: IAA and GA are essential growth regulator and induces plant growth and development under stress condition. This study demonstrated that application of IAA 100 and 250 mM and GA 100 and 250 mM significantly increased the number of leaves, number of branches, and diameter of stem in *J. curcas* in response to watrlogged and drought stress (Fig. 5). The first data was initially collected after one week and the 2nd and 3rd data was collected after 9th and 17th week of hormonal application. Our results showed that both the stresses reduced leaves number, branches number and stem development compared with control plants. However, IAA and GA noticeably increased number of leaves number of branches, and dimeter of stem during both the stresses compared with their respective stress. For instance, WL+IAA 250 mM plants increased number of leaves 81% and WL+GA 250 mM plants increased 190% under waterlogging compared with waterlogged stressed plants after 17 months of hormonal application (Fig. 5A). While on the other hand, DS+IAA 250 mM plants increased leaves number 33% and DS+GA 250 mM plants increased 80% under drought stress compared to drought stressed plants after 17 months of hormonal application (Fig. 5A). These results demonstrated that, GA greatly enhanceed leaves number than the IAA under both the stresses. Similarly, the branches were reduced 30% and 19% after 17 weeks in waterlogging and drought stress compared to control plants (Fig. 5B). The branches number were increased 74% in the DS+GA 250 mM plants followed by WL+GA 250 mM plants (57%) and DS+GA 100 mM (49%) compared to their respective stressed plants after 17 weeks of hormonal application. Furthermore, DS+GA 250 mM plants and DS+GA 100 mM plants enhanced the stem diameter 55% and 44% respectively, compared to drought stressed plants while, WL+GA 250 mM plants and WL+GA 100 mM plants increased stem diameter 44% and 35% respectively, compared to waterlogged stressed plants (Fig. 5C). Form the results it was found that, with the increase of hormones concentration, the leaves, branches and stem development increased under both the stresses. However, the GA increased the morphological parameters more than the IAA. Although, in some cases the morphological parameters were affected even under the hormonal supplementation compared to control plants, but compared to their respective stress they were significantly enhanced due to exogenous hormonal application. All the morphological investigation showed that waterlogging more strongly effected *J. curcas* compared to drought stress.

IAA and GA promote *J. curcas* proximate composition contents during waterlogging and drought stress: In the present study, we evaluated the effect of IAA and GA on proximate composition contents (protein, carbohydrate, fat, moisture, and ash) of *J. curcas* plants under waterlogging and drought stress. After 17 weeks of the study, the samples for the proximate composition contents analysis were taken. The results indicated that waterlogging significantly inhibited proximate compositions of *J. curcas* while, drought stress showed irregular effect on proximate composition. The protein, carbohydrates, moisture and ash contents were significantly reduced 15, 22, 23, and 34% respectively in waterlogged plants compared with control plants (Fig. 6). The IAA and GA mostly enhanced the accumulation of all the components of proximate composition under both the waterlogging and drought stress. The results indicated that GA greatly enhanced the compositions compared to IAA hormones. The percentage of proximate compositions were increased with the increasing the concentration of both the hormones. GA 250 mM increased protein, carbohydrates, fat, moisture and ash about 80, 94, 58, 78, and 145% respectively, during waterlogging stress compared with waterlogged plants. While on the other hand, GA 250 mM increased protein (87%), carbohydrates (117%), fat (58%), moisture (50%) and ash (123%) contents under drought stress compared with drought stressed plants. Likewise, IAA 250 mM significantly increased all the proximate compositions in response to waterlogging compared with waterlogged stressed plants. However, IAA 250 mM did not promoted proximate compositions in response to drought stress. These results showed that IAA was not efficiently involved in the regulation of proximate composition accumulation during drought stress while GA was involved in regulation of these compositions under both the stresses.

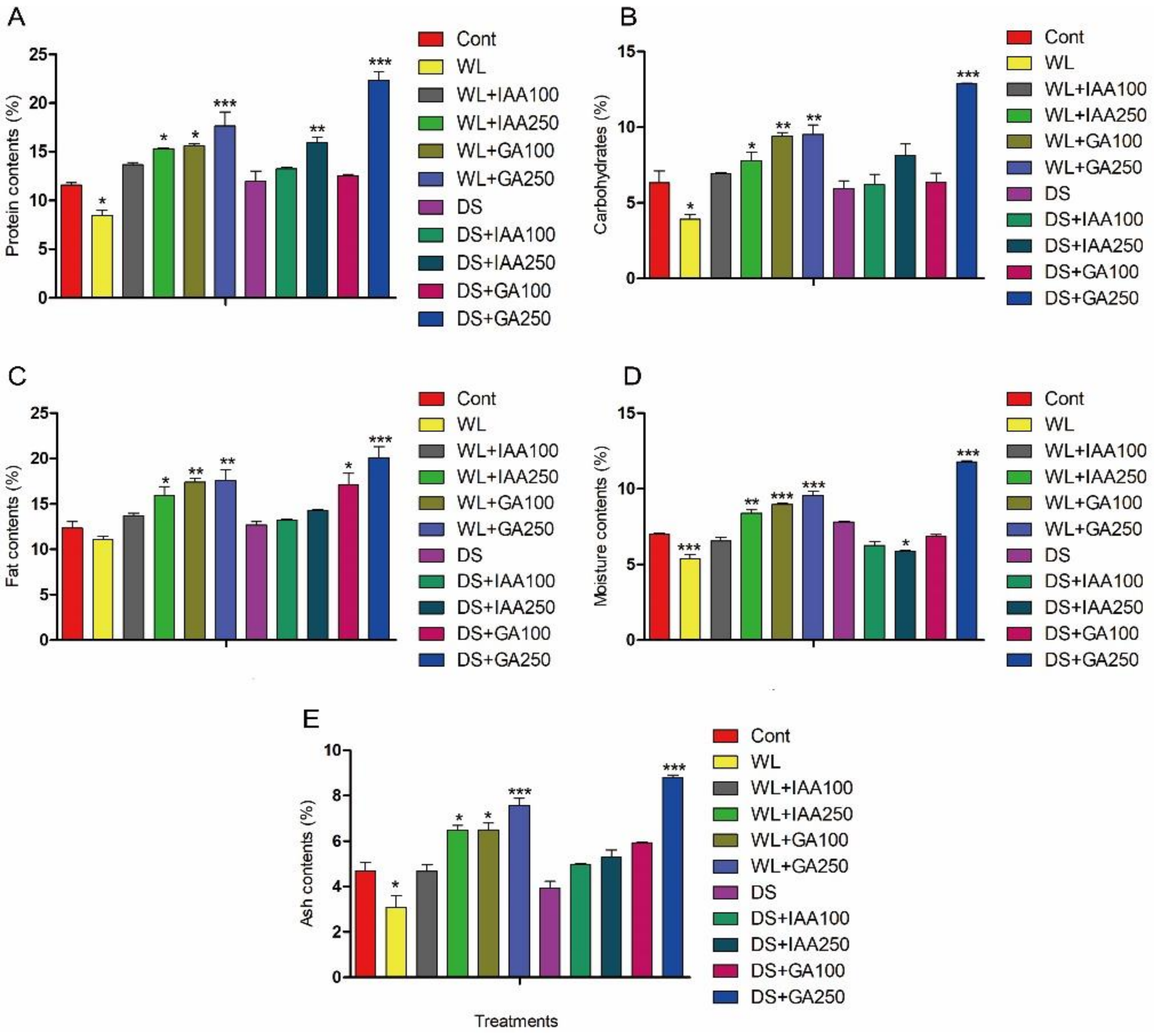


Fig. 6. IAA and GA regulate proximate composition contents of *J. curcas* during waterlogging and drought stress. (A) Shows protein contents, (B) shows carbohydrate contents, (C) shows fat contents, (D) shows moisture contents, and (E) shows ash contents. Graphs show mean ± standard deviation, and asterisks show significant differences (* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$) according to one-way ANOVA with Bonferroni post hoc tests. Waterlogged and drought stresses were compared with control plants while hormone applied plants under stress were compared with their respective stressed plants.

Discussion

We have evaluated *Jatropha curcas* growth pattern, susceptibility and resistance to waterlogging and drought stress using exogenous IAA and GA hormones. We found that exogenous IAA and GA promoted plant growth under both the stresses via regulation of physiological, morphological and biochemical parameters. While both, waterlogging and drought stress reduced these parameters. Generally, *J. curcas* survived in both the stresses. However, compared to drought, it was susceptible to waterlogging. Our study demonstrated that *J. curcas* seedlings or young plants are highly susceptible to frost condition and could not survive if exposed to frost continuously. However, the low temperature caused severe damage to mature plants and significantly affected leaves, flowers and fruits (Fig. 3).

IAA is an essential plant growth promoter and one of the most active form of auxin. Present study showed that exogenous application of IAA significantly increased shoot length of the *J. curcas* under waterlogging and drought stress compared to non-treated waterlogged and drought stressed plants (Fig. 4A). However, IAA did not affected other parameters such as, root length, shoot fresh and dry weight and root fresh and dry weight under both the stress conditions. Recently it is found that, IAA act as a key enhancer of shoot during flooding condition (Zhao *et al*., 2021). In our study, waterlogging significantly inhibited root length, shoot fresh and dry weight and root fresh weight as compared to control plants. While on the other hand, drought stress only reduced shoot length, shoot fresh and dry weight significantly, compared to control plants. These results showed that, waterlogging had more severe effects on growth and development of *J. curcas* plant compared with drought stress. In this regard, previous researchers stated that, flooding modulated almost all phenotypic parameters of plant (Whitlow and Harris, 1979). In general, flooding effects started when the molecular oxygen concentration reduced in the rhizosphere to that level where it was difficult to maintain the aerobic respiration in root tissue (Younis *et al.*, 2003). Waterlogging inhibit plant development by several ways such reduction of oxygen availability, inhibition of photosynthesis, and reduction of dry matter (Younis *et al.*, 2003). The reduction in dry matter accumulation in our study might be attributed to reduction of water uptake and to inhibition of rate of photosynthesis. There are several reports that, in waterlogging, plant induces endogenous biosynthesis of IAA such as in *Vigna sinensis*, *Zea mays* and *Helianthus annuus* (Phillips, 1964; Younis *et al.*, 2003). Recently, it was claimed that IAA producing bacteria (*Klebsiella variicola*) enhanced growth and development of soybean plants via IAA accumulation under flooding stresses (Kim *et al.*, 2017). Further, our study showed that IAA greatly induced *J. curcas* leaves, branches, stem diameter, and proximate compositions under waterlogging compared with waterlogged non-treated plants (Fig. 5, 6). Like other growth promoters, IAA is efficiently involved in the regulation of several physiological, metabolism, growth and development processes. Studies have shown that IAA is mainly involved in root/shoot development and several other cellular and physiological processes such as, cell expansion, apical dominance, and leaf and embryo development (Sachs, 2005; Tromas *et al.*, 2009; Jurado *et al.*, 2010). Exogenous application of IAA increased plant height, blades number, leaf area, and shoot fresh weight in *Cleistocalyx operculatus* and *Syzygium jambos* plants under waterlogging stress (Cisse *et al.*, 2022). The increase in plant biomass of *J. curcas* under waterlogging is possibly due to high rate of photosynthesis as the exogenous IAA increases rubisco and photosynthesis rate (Amoanimaa-Dede *et al.*, 2022). Waterlogging inhibit growth of the main root however, the adventitious root facilitate oxygen supply, which results into plant growth and development. Our study predicted that, IAA plays an essential role in development of adventurous root in waterlogging condition which results into improvement of plant biomass. In the previous study, it was found that IAA promoted adventurous roots in Arabidopsis plant under flooded conditions (da Costa *et al.*, 2020).

Our study further exposed the role of IAA in *J. curcas* under drought stress. The IAA enhanced plant length, shoot dry weight, leaves number, branches number, and stem diameter under drought stress compared with drought stressed non-treated plants (Figs. 4, 5). While in contrast to waterlogging, our results showed that IAA had non-significant effect on proximate composition biosynthesis under drought stress (Fig. 6). Drought stress is harmful to the growth and development of plant by affecting their physiological, morphological, biochemical and genetic resources that results to diminishes productivity (Khan and Mazid, 2018). Drought drastically affect the proper functioning of plant by disturbing the water use potential and causing oxidative stress which results into protein, lipid and nucleic acid degradation (Lipiec *et al.*, 2013). Generally, drought stress causes oxidative stress due to over-accumulation of reactive oxygen species (ROS) which results into reduced growth and biomass accumulation. Previous studies show that exogenous application of IAA increases drought stress tolerance in *Trifolium repens* by inducing ABA, JA related genes and inhibiting senescence genes (Zhang *et al.*, 2020). The IAA producing bacteria also enhance the plant growth in stress condition because of IAA production (Khan *et al.*, 2021). In *Withania somnifera*, IAA application reduced the accumulation of ROS through increasing the antioxidant activities (Takshak & Agrawal, 2017). There is lack of data about mitigation of drought stress in *J. curcas* plant via IAA application. However, previously we found that IAA application significantly enhance *J. curcas* growth and development (Jan *et al.*, 2022). In white clover, IAA application significantly increased relative water contents, stem dry weight, and chlorophyll contents under drought stress (Zhang *et al.*, 2020). However, in our study, we found that dry weight was increased while the water content was reduced in the IAA treated plants under drought stress.

GA is one of the key growth promoter and regulate many plant growth attributes such as, seed germination, stem elongation, leaf expansion, flower and fruit development, stomatal function regulation, root to shoot communication, bud formation, and root development (Hu *et al.*, 2017; Khan *et al.*, 2018). In this study, we found that the application of GA promoted growth parameters and proximate composition level of *J. curcas* under waterlogging and drought stress. Studies described that, exogenous GA provide resistance in plant under different stresses especially against hypoxia, waterlogging and pathogen (Dordas, 2015; Fan *et al.*, 2017; Gupta *et al.*, 2017; Asif *et al.*, 2022). There is lack of data regarding effect of GA on *J. curcas* stress tolerance under waterlogging and drought stress however, GA application enhance the growth of soybean under flooding condition (Khan *et al.*, 2018). The continuous exposure of *J. curcas* to the waterlogging condition tend to develop the defensive strategy to overcome the waterlogging induced oxidative stress. It was suggested that, GA application enhanced endogenous GA accumulation, resulting into mitigation of waterlogging stress and promotion of plant growth and development (Khan *et al.*, 2018). Our results showed that GA enhanced leaves and branches number of *J. curcas* under both the stresses. Previous study showed that, GA application induced the lateral branches of *J. curcas* and the branches were increased with the increase of GA concentration (Koorneef *et al.*, 1985). Sometime, GA reduces lateral branches depending on the plant species. For instance, GA inhibited lateral branches formation in *Pisum sativum* while on the other hand, the expression of GA biosynthesis gene induced tiller/branches of *Paspalum distichum* and *Populus tremula,* suggesting that GA regulated branching of these species (Scott *et al.*, 1967; Agharkar *et al.*, 2007; Mauriat *et al.*, 2011; Zawaski & Busov, 2014). Previously we reported that in normal condition, GA increased stem diameter and proximate composition level but here we witnessed the same result under both the waterlogging and drought stress. These results suggested that, exogenous application significantly induced *J. curcas* growth and development during both the stress conditions.

Conclusion

This study concluded that, *J. cusrcas* is susceptible to low temperature and waterlogging while efficiently resistant to drought stress. The study further validated that, application of IAA and GA hormones promoted *J. curcas* growth under waterlogging and drought stress. While both the hormone fail to protect *J. curcas* from low temperature and frost condition. Between both the hormones, GA showed best results than IAA and promoted almost all the growth parameters under both the stresses. Furthermore, waterlogging more severely effected *J. curcas than* drought stress. The present study open a key research area for the future study to explore the molecular mechanism through which hormones promotes *J. curcas* succession in waterlogging and drought stress.

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References

Abou Kheira, A.A. and N.M. Atta. 2009. Response of *Jatropha curcas* L., to water deficits: Yield, water use efficiency and oilseed characteristics. *Biom. Bioenergy*, 33(10): 1343-1350.

Achten, W.M., L. Verchot, Y.J. Franken, E. Mathijs, V.P. Singh, R. Aerts and B. Muys. 2008. Jatropha bio-diesel production and use. *Biom. Bioenergy*, 32(12): 1063-1084.

Agharkar, M., P. Lomba, F. Altpeter, H. Zhang, K. Kenworthy and T. Lange. 2007. Stable expression of atga2ox1 in a low-input turfgrass (*Paspalum notatum* flugge) reduces bioactive gibberellin levels and improves turf quality under field conditions. *Plant Biotech. J.*, 5(6): 791-801.

Al-Busaidi, A., M. Ahmed and J. Chikara. 2012. The impact of heat and water stress conditions on the growth of the biofuel plant *Jatropha curcas*. *Int. J. Environ. Stud*., 69(2): 273-288.

Ali, S., S.K. Park and W.C. Kim. 2018. The pragmatic introduction and expression of microbial transgenes in plants. *J. Microbiol. Biotech.*, 28(12): 1955-1970.

Almazan, A.M. and S.O. Adeyeye. 1998. Fat and fatty acid concentrations in some green vegetables. *J. Food Comp. Anal.,* 11(4): 375-380.

Amoanimaa-Dede, H., C. Su, A. Yeboah, H. Zhou, D. Zheng and H. Zhu. 2022. Growth regulators promote soybean productivity: A review. *Peer J.,* 10: e12556.

Anfang, M. and E. Shani. 2021. Transport mechanisms of plant hormones. *Curr. Opin. Plant Biol*., 63: 102055.

Arbona, V., Z. Hossain, M.F. López-Climent, R.M. Pérez-Clemente and A. Gómez-Cadenas, 2008. Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. *Physiol. Plant.*, 132(4): 452-466.

Asif, S., Y.H. Jang, E.G. Kim, R. Jan, S. Asaf, M. Aaqil Khan, M. Farooq, N. Kim, I.J. Lee and K.M. Kim. 2022. The role of exogenous gibberellic acid and methyl jasmonate against white-backed planthopper (*Sogatella furcifera*) stress in rice (*Oryza sativa* L.). *Int. J. Mol. Sci*., 23(23): 14737.

Boyer, J.S. 1982. Plant productivity and environment. *Science*, 218(4571): 443-448.

Cisse, E.H.M., J. Zhang, D.D. Li, L.F. Miao, L.Y. Yin and F. Yang. 2022. Exogenous aba and iaa modulate physiological and hormonal adaptation strategies in cleistocalyx operculatus and syzygium jambos under long-term waterlogging conditions. *BMC Plant Biol*., 22(1): 1-20.

da Costa, C.T., R. Offringa and A.G. Fett-Neto. 2020. The role of auxin transporters and receptors in adventitious rooting of arabidopsis thaliana pre-etiolated flooded seedlings. *Plant Sci*., 290: 110294.

Divakara, B., H. Upadhyaya, S. Wani and C.L. Gowda. 2010. Biology and genetic improvement of *Jatropha curcas* L.: A review. *Appl. Energy*, 87(3): 732-742.

Dordas, C. 2015. Nitric oxide and plant hemoglobins improve the tolerance of plants to hypoxia. In: Nitric oxide action in abiotic stress responses in plants (pp: 115-128). Springer, Cham.

Drew, M.C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., 48(1): 223-250.

Du, H., N. Wu, J. Fu, S. Wang, X. Li, J. Xiao and L. Xiong. 2012. A gh3 family member, osgh3-2, modulates auxin and abscisic acid levels and differentially affects drought and cold tolerance in rice. *J. Exp. Bot*., 63(18): 6467-6480.

Fan, X., M. Naz, X. Fan, W. Xuan, A.J. Miller and G. Xu. 2017. Plant nitrate transporters: From gene function to application. *J. Exp. Bot.*, 68(10): 2463-2475.

Francis, G., R. Edinger and K. Becker. 2005. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: Need, potential and perspectives of jatropha plantations. In: *Natural resources forum* (pp: 12-24). Wiley Online Library, United Nations.

Ghosh, A., J. Chikara, D. Chaudhary, A.R. Prakash, G. Boricha and A. Zala. 2010. Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jatropha curcas*. *J. Plant Growth Reg*., 29(3): 307-315.

Gimeno, V., J.P. Syvertsen, I. Simón, M. Nieves, L. Díaz-López, V. Martínez and F. García-Sánchez. 2012. Physiological and morphological responses to flooding with fresh or saline water in *Jatropha curcas*. *Environ. Exp. Bot.*, 78: 47-55.

Groot, S. and C. Karssen. 1987. Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. *Planta*, 171(4): 525-531.

Gupta, K.J., C.P. Lee and R.G. Ratcliffe. 2017. Nitrite protects mitochondrial structure and function under hypoxia. *Plant Cell Physiol.*, 58(1): 175-183.

Hu, Y.X., Y.B. Tao and Z.F. Xu. 2017. Overexpression of jatropha gibberellin 2-oxidase 6 (jcga2ox6) induces dwarfism and smaller leaves, flowers and fruits in arabidopsis and jatropha. *Front. Plant Sci*., 8: 2103-2115.

Hui, W., S. Yang, H. Chen, S. Li, C. Peng, G. Wu and X. Chen. 2016. Female and male flower bud differentiation of *Jatropha curcas* L. induced by gibberellin. *J. Nanjing Forestry Uni.* (*Natural Sciences Edition*), 40(6): 174-180.

Islam, M.R., M.M. Rahman, M. Mohi-Ud-Din, M. Akter, E. Zaman, S.S. Keya, M. Hasan and M. Hasanuzzaman. 2022. Cytokinin and gibberellic acid-mediated waterlogging tolerance of mungbean (*Vigna radiata* L. Wilczek). *Peer J*., 10: e12862.

Jackson, M.B., W.J. Davies and M.A. Else. 1996. Pressure–flow relationships, xylem solutes and root hydraulic conductance in flooded tomato plants. *Ann. Bot.*, 77(1): 17-24.

Jan, R., M. Khan, M. Adnan, S. Asaf, S. Asif, K.-M. Kim and W. Murad. 2022. Exogenous phytohormones and fertilizers enhance *Jatropha curcas* L., growth through the regulation of physiological, morphological, and biochemical parameters. *Plants,* 11(24): 3584-3598.

Jung, H., S. Ali, J.Y. Kim and W.C. Kim. 2018. Transgenic arabidopsis expressing acds gene of pseudomonas veronii-kj alleviate the adverse effects of salt and water-logging stress. *Plant Breed. Biotech*., 6(3): 221-232.

Jurado, S., Z. Abraham, C. Manzano, G. López-Torrejón, L.F. Pacios and J.C. Del Pozo. 2010. The arabidopsis cell cycle f-box protein skp2a binds to auxin. *The Plant Cell*, 22(12): 3891-3904.

Kende, H. and J. Zeevaart. 1997. The five" classical" plant hormones. *The Plant Cell,* 9(7): 1197-1210.

Khalid, K.A. 2006. Influence of water stress on growth, essential oil, and chemical composition of herbs [*Ocimum* sp.]. *Int. Agrophysics.,* 20(4): 289-296.

Khan, K. and M. Mazid. 2018. Chickpea responses to application of plant growth regulators, organics and nutrients. *Adv. Plants Agri. Res*., 8: 259-273.

Khan, M.A., M. Hamayun, A. Iqbal, S.A. Khan, A. Hussain, S. Asaf, A.L. Khan, B.W. Yun and I.J. Lee. 2018. Gibberellin application ameliorates the adverse impact of short-term flooding on *Glycine max* L. *Biochem. J*., 475(18): 2893-2905.

Khan, M.A., A.A. Sahile, R. Jan, S. Asaf, M. Hamayun, M. Imran, A. Adhikari, S.M. Kang, K.M. Kim and I.J. Lee. 2021. Halotolerant bacteria mitigate the effects of salinity stress on soybean growth by regulating secondary metabolites and molecular responses. *BMC Plant Biol*., 21(1): 1-15.

Kim, A.Y., R. Shahzad, S.M. Kang, C.W. Seo, Y.G. Park, H.J. Park and I.J. Lee. 2017. Iaa-producing klebsiella variicola ay13 reprograms soybean growth during flooding stress. *J. Crop Sci. Biotech*., 20(4): 235-242.

Koorneef, M., A. Elgersma, C.V. Hanhart, E. van Loenen-Martinet, L. Van Rijn and J. Zeevaart. 1985. A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol. Plant*., 65(1): 33-39.

Kozlowski, T. 1997. Responses of woody plants to flooding and salinity. *Tree Physiol*., 17(7): 490-490.

Latimer, G. 2016. Official methods of analysis of aoac international. Aoac international, rockville, MD, USA.

Lipiec, J., C. Doussan, A. Nosalewicz and K. Kondracka. 2013. Effect of drought and heat stresses on plant growth and yield: A review. *Int. Agrophy*., 27(4): 463-477.

Mæhre, H.K., L. Dalheim, G.K. Edvinsen, E.O. Elvevoll and I.J. Jensen, 2018. Protein determination-method matters. *Foods*, 7(1): 5.

Maes, W.H., W.M. Achten, B. Reubens, D. Raes, R. Samson and B. Muys. 2009. Plant–water relationships and growth strategies of *Jatropha curcas* L. Seedlings under different levels of drought stress. *J. Arid Enviro.*, 73(10): 877-884.

Marcel, A. and M.J. Bievenu. 2012. Proximate, mineral and phytochemical analysis of the leaves of *H. Myriantha* and *Urera trinervis*. *Pak. J. Biol. Sci*., 15(11): 536-541.

Mauriat, M., L.G. Sandberg and T. Moritz. 2011. Proper gibberellin localization in vascular tissue is required to control auxin-dependent leaf development and bud outgrowth in hybrid aspen. *The Plant J*., 67(5): 805-816.

Mielke, M.S., A.A.F. de Almeida, F.P. Gomes, M.A.G. Aguilar and P.A.O. Mangabeira. 2003. Leaf gas exchange, chlorophyll fluorescence and growth responses of genipa americana seedlings to soil flooding. *Environ. Exp. Bot*., 50(3): 221-231.

Moon, Y.S. and S. Ali. 2022. A fruitful decade of bacterial acc deaminase biotechnology: A pragmatic approach towards abiotic stress relief in plants. *Theor. Exp. Plant Physiol.*, 34(2): 109-129.

Ogunwole, J., D. Chaudhary, A. Ghosh, C. Daudu, J. Chikara and J. Patolia. 2008. Contribution of *Jatropha curcas* to soil quality improvement in a degraded indian entisol. *Acta Agri. Scand.*, 58(3): 245-251.

Openshaw, K. 2000. A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biom. Bioenergy*, 19(1): 1-15.

Pan, B.Z., Y. Luo, L. Song, M.S. Chen, J.L. Li and Z.F. Xu. 2016. Thidiazuron increases fruit number in the biofuel plant *Jatropha curcas* by promoting pistil development. *Ind. Crops Prod.*, 81: 202-210.

Pan, B.Z. and Z.F. Xu. 2011. Benzyladenine treatment significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *J. Plant Growth Reg*., 30(2): 166-174.

Paquot, C. and A. Hautfenne, 1987. Standard methods for the analysis of oils, fats and derivatives. *Anal. Chimica Acta*, 201: 371.

Pérez-Vázquez, A., G. Hernández-Salinas, C. Ávila-Reséndiz, O. Valdés-Rodríguez, F. Gallardo-López, E. García-Pérez and O. Ruiz-Rosado. 2013. Effect of the soil water content on jatropha seedlings in a tropical climate. *Int. Agrophy*., 27(3): 351-357.

Phillips, I. 1964. Root-shoot hormone relations i. The importance of an aerated root system in the regulation of growth hormone levels in the shoot of *Helianthus annuus*. *Ann. Bot.*, 28(1): 17-35.

Pociecha, E., J. Kościelniak and W. Filek. 2008. Effects of root flooding and stage of development on the growth and photosynthesis of field bean (*Vicia faba* L. Minor). *Acta Physiol. Plant.*, 30(4): 529-535.

Qin, F., K. Shinozaki and K. Yamaguchi-Shinozaki. 2011. Achievements and challenges in understanding plant abiotic stress responses and tolerance. *Plant Cell Physiol*., 52(9): 1569-1582.

Sachs, T. 2005. Auxin's role as an example of the mechanisms of shoot/root relations. *Plant Soil.*, 268(1): 13-19.

Said-Al, A.H., E. Omer and N. Naguib. 2009. Effect of water stress and nitrogen fertilizer on herb and essential oil of oregano. *Int. Agrophy*., 23(3): 269-275.

Sarker, B.C., M. Hara and M. Uemura. 2005. Proline synthesis, physiological responses and biomass yield of eggplants during and after repetitive soil moisture stress. *Sci. Hort*., 103(4): 387-402.

Scott, T.K., D.B. Case and W.P. Jacobs. 1967. Auxin-gibberellin interaction in apical dominance. *Plant Physiol*., 42(10): 1329-1333.

Silva, E.d., R. Ribeiro, S. Ferreira-Silva, R. Viégas and J. Silveira. 2010. Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. *J. Arid Environ*., 74(10): 1130-1137.

Takshak, S. and S. Agrawal. 2017. Exogenous application of iaa alleviates effects of supplemental ultraviolet-b radiation in the medicinal plant withania somnifera dunal. *Plant Biol*., 19(6): 904-916.

Tromas, A., N. Braun, P. Muller, T. Khodus, I.A. Paponov, K. Palme, K. Ljung, J.Y. Lee, P. Benfey and J.A. Murray. 2009. The auxin binding protein 1 is required for differential auxin responses mediating root growth. *PLoS One.,* 4(9): e6648.

Valdes-Rodriguez, O.A., O. Sanchez-Sanchez, A. Perez-Vazquez and R. Ruiz-Bello. 2011. Soil texture effects on the development of jatropha seedlings–mexican variety 'piñón manso'. *Biom. Bioenergy.,* 35(8): 3529-3536.

Verma, K.K., M. Singh, R.K. Gupta and C.L. Verma. 2014. Photosynthetic gas exchange, chlorophyll fluorescence, antioxidant enzymes, and growth responses of *Jatropha curcas* during soil flooding. *Turk. J. Bot.*, 38(1): 130-140.

Verma, K.K., M. Singh and C.L. Verma. 2012. Developing a mathematical model for variation of physiological responses of *Jatropha curcas* leaves depending on leaf positions under soil flooding. *Acta Physiol. Plant*., 34(4): 1435-1443.

Visser, E.J. and L.A. Voesenek. 2005. Acclimation to soil flooding–sensing and signal-transduction. *Plant Soil*, 274(1): 197-214.

Wang, Y., J. Zhao, W. Lu and D. Deng. 2017. Gibberellin in plant height control: Old player, new story. *Plant Cell Rep.,* 36(3): 391-398.

Whitlow, T.H. and R.W. Harris. 1979. Flood tolerance in plants: A state-of-the-art review (p. 161). Waterways Expreiment Station.

Younis, M., O. El-Shahaby, M.M.N. Alla and Z. El-Bastawisy. 2003. Kinetin alleviates the influence of waterlogging and salinity on growth and affects the production of plant growth regulators in *Vigna sinensis* and *Zea mays*. *Agronomie,* 23(4): 277-285.

Zawaski, C. and V.B. Busov. 2014. Roles of gibberellin catabolism and signaling in growth and physiological response to drought and short-day photoperiods in populus trees. *PLoS One*, 9(1): e86217.

Zhang, Y., Y. Li, M.J. Hassan, Z. Li and Y. Peng. 2020. Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. *BMC Plant Biol.*, 20(1): 1-12.

Zhao, Y., W. Zhang, S.F. Abou-Elwafa, S. Shabala and L. Xu. 2021. Understanding a mechanistic basis of aba involvement in plant adaptation to soil flooding: The current standing. *Plants*, 10(10): 1982-1997.

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