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Effect of hormones on seed germination of lemon seeds

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Abstract
Grapefruit, sweet orange, lemon, lime, mandarin, and other citrus fruits are among the world's most important tree fruit crops. Seeds are used to propagate the majority of Citrus rootstock species. The goal of this study was to determine how different growth regulators affected seed germination. The seeds were soaked in various quantities of growth regulators such as Indole-3-Acetic Acid (Auxin) and 6-benzyl aminopurine (Cytokinin) to observe how they affected germination. It was found that when the increasing concentration of auxin (ppm), root size also increased at that time and variation seen in shoot size. So auxin is more affected on root size compare to shoot size. When increasing Cytokinin (ppm) concentration, shoot size decrease at that time and variation seen in root size. Cytokinin is more affected by shoot size in comparison to root size. In the different ratio of Auxin and Cytokinin, root size and shoot size is different. While using Auxin Cytokinin ratio positive results were obtained in both root and shoot growth when compared to the control. This is the maximum height and positive experimental observations based on all of the above observations.

Keywords: citrus, growth regulator, seed germination, lemon

Introduction

Grapefruit, sweet orange, lemon, lime, mandarin, and other citrus fruits are among the world's most important tree fruit crops. Seeds are used to propagate the majority of Citrus rootstock species. Seed germination is a component wherein morphological and physiological changes bring about the enactment of the incipient organism. Before germination, the seed ingests water, bringing about the extension and stretching of the incipient seed organism. The cycle of seed germination is completed when the radicle has surpassed the covering seed layers [1]. Many scientists have studied the processes involved in seed germination and how plant hormones affect them in a variety of plant families [1, 2]. It is thought that seeds contain proteins called globulins and prolamins that become more abundant while seed maturation, particularly during the middle and last stages when seeds become rich in nitrogen. The proteins are located in the cell membrane or other places in the seed. During the protein translocation process, only a tiny amount of protein is converted into other products. As a result of activating enzymes like proteinase, storage proteins are mobilized [3].

Seed Germinations

Plants use regeneration to maintain and extend their populations over time [4]. The season in which a seedling germinates has a direct relationship with its chances of survival. Because climatic circumstances at the time of necessary dispersal are often inadequate for seedling survival, many temperate species have mechanisms to prevent germination immediately after shedding. Seed dormancy is a cryptobiotic state that functions as a growth halting adaptation mechanism, often offering a selective advantage in dispersion and abundance [5]. Many species' seedling growth has been improved by pre-sowing seed treatments with growth agents such gibberellic acid [6, 7]. Different hormones may now be extracted from plant tissue and their identification, quantity, and quality tested using particular techniques. As a result, both quantitative and qualitative hormone variation can be observed, linked to specific growth patterns and responses to environmental factors. Seed germination and seedling establishment were particularly vulnerable to drought stress, which resulted in lower seedling germination and establishment. Increase seed performance by the employment of various methods of promotion can reach the end aim of boosting seed and forage proceeds, which is very successful. Gibberellic acid is a plant growth hormone that plays a critical function in the control of seed germination. Phytohormones' discovery has been a massive boost to agriculture and related fields. Exogenous application of phytohormones and other mechanical, chemical, and physiological metlux1 has aided in increasing flower, fruit, and other agriculture commodities production. Exogenous hormones are known to control seed germination and seedling growth [8]. More than 126 different GAs have been isolated from various plants and plant organs as of today. Seedlings are the most fragile stage of a plant's life cycle, and when and where seedling growth begins is determined by germination [9]. Gibberellic acid increased the germination rate of Barley and lettuce seed under salt-stress treatment [10, 11]. Phytohormone, in general, was found to regulate the transport of ions in plants [12]. Stimulatory effect of GA (10-14M) on K+ Transport in excised cotyledons of cucumber was reported [13]. Walker and Douglas (1983) observed NaCl-salinity stress GA and Kn together increased germination percentage in lettuce seeds [14].

Seed germination in citrus

Citrus, which comprises sweet orange, mandarin, lemon, lime, grapefruit, and others, is one of the world's most significant commercial fruit crops [15]. Seeds are used to propagate the majority of Citrus rootstock species. When desirable cultivars are propagated by budding or grafting on a rootstock, characters are duplicated through this genetically identical plant. Citrus seed germination is typically sluggish and unpredictable. Citrus seed germination can be slowed for various reasons, including the presence of growth inhibitors and the seed coat's physical barrier to radicle protrusion [16]. Gibberellins have been shown to enhance the germination of a variety of seeds in diverse ways. Several researchers discovered that gibberellic acid (GA) enhances germination rates (the speed with which seeds germinate), for example, in sweet oranges. [17], Cleopatra mandarin and sour orange [18], peach (*Primus persica* L.) [19], or final percentage germination as seen in *Solanum incanum* and pawpaw (*Carica papaya* L.) [20]. However, it is unknown if GA solutions penetrated the seeds; GA cannot reach the embryo in some Labiate species due to seed coat impermeability [21]. It has been suggested that applying GA at a concentration of 10-15ppm to trifoliate oranges, empress lemons and mandarins (*C. reticulate* Blanco) can speed up germination [22], increase the germination percentage in trifoliate orange (Button *et al*., 1971). However, a similar concentration did not affect germination of Troyer orange (*Poncirus trifoliaie* x *C. Sinemis* Osbeck) or sweet orange (*C. sinensis*) [17, 23]. A concentration of 500 ppm has been reported as having improved the germination of sweet lime (*C. limettioides* Tan.) [24], as well as 1000 ppm that of sweet orange [17]. Before GA treatment of sweet lime (*C. limettioides* Tan.) seeds, Achitus and Menda1 (1973) utilized citric acid or EDTA (ethylene diamine tetraacetic acid). These treatments increased the rate of germination sowing due to greater absorption of water and GA3 solutions. Fresh citrus seeds do not germinate quickly, and dried seeds germinate even slowly [24].

The purpose of this study was to look at the effects of several growth regulators such as Indole-3-Acetic Acid (Auxin) and 6-benzyl aminopurine (Cytokinin) in various concentrations to evaluate their effect on the rate of germination and find percentage germination of lemon seeds (*Citrus limon* (L.) Burm. f.; Family-Rutaceae). These species' seed and seed coat morphological traits may vary significantly, affecting their germination performance.

Hormone

Plant growth regulators are substances that, when administered in very small amounts, regulate plant development. Many studies have found that using growth regulators improves plant growth and production. GA boosted the quantity of blossoms per plant and the length of the gems. [25, 26]. GA3 enhanced bud development and stem elongation, according to Kabar (1989); however, the best results are obtained when GA3 is used in conjunction with kinetin [10].

Auxin

By widening leaves and enhancing photosynthetic activity in plants, IAA influences plant growth. It also promotes carbohydrate translocation during their production [27].

Cytokinins

Cytokinins enhanced the cell expansion in soybean [28], Morning glory kinetin reduced shoot length but increased fresh weight by increasing stern diameter, whereas kinetin reduced shoot length but increased fresh weight by increasing stern diameter [29] and Okra [30]. Some reports indicate that kinetin combined with GA3 enhanced germination and seedling growth in chickpea [31].

Citrus trees are evergreen trees that yield fragrant, flavorful, and juicy fruits in various shapes and sizes (from round to rectangular). It has epics, or flayed skin or rind that is challenging, robust, and bright in colour, ranging from green to yellow, and protects the fruits from injury. The glands contain essential oil, which is responsible for the fruit's distinctive citrus scent. It is made up of a thick, spongy white mesocarp or albedo that, along with the epicarp, forms the fruit's pericarp or peel. The pulp is split into discrete segments or juice sacs (with or without seeds, depending on variety) by a thick radical film or endocarp on the inside. This apple section is high in soluble sugars, ascorbic acid, pectin, fibres, organic acids, and potassium salt, contributing to the fruit's distinctive citrine flavour. Although the actual origin of citrus fruits is unknown, most academics believe it originated in Southeast Asia [32]. Later, the Spaniards brought the citrus fruits to America, notably to Mexico, Florida, Brazil, and California, where we now have the world's most extensive orange orchards. Citrus fruits are high in phytonutrients, essential for both illness prevention and health enhancement [33, 34]. Citrus fruits have similar dietary and medicinal effects due to their phytonutrient concentration. These second metabolites include several classes such as terpenoids, flavonoids and phenolics compounds with diverse chemical structures and biological activities and exist widely in citrus fruits.

Material and Methods

Collection of lemon seed

The lemon seed (*Citrus limon* (L.) Burm. f.; Family-Rutaceae) was collected from fresh lemon fruits which are readily gets from botanical garden of Veer Narmad South Gujarat University, Surat.

Preparation of hormone stock solution

The effects of different growth regulators such as Indole-3-Acetic Acid (Auxin), 6-benzyl aminopurine (Cytokinin) and mixture of Auxin-Cytokinin in various doses were studied using lemon seeds and the stock solutions were made as follows.

Auxin (IAA)

Auxin stock standard was prepared and afterwards 10 ppm, 30 ppm, and 50 ppm auxin were prepared from the stock solution.

Cytokinin (BAP)

Prepared a Cytokinin stock solution, then produced 10 ppm, 30 ppm, and 50 ppm Cytokinin from it.

Auxin-Cytokinin mixture

Prepared 1:1, 1:2 and 2:1 ratio of Auxin-Cytokinin mixtures for treatment.

Procedure

Preparation of soil Sample

To prepare potting soil, combine vemiculite and peat. Leaf mould and compost, both organic materials that give a variety of nutrients, are also included in the potting mix. Combine all of the ingredients in a large mixing bowl.

Water was applied to the soil for half an hour before sowing the lemon seed to hydrate it.

Preparation of seed
In control, 25 ml distilled water poured into the seed of control petiolate for three hours and 25 ml hormone added to the other 9 Petri plates of seed for three hours for hormone treatment. These three Petri plates for the ratio of 1:1, 1:2, 2:1 and three Petri plates for 10ppm, 30ppm, 50ppm auxin and other three Petri plates for ten ppm, 30 ppm and 50 ppm cytokinin. After priming, seeds were rinsed in distilled water and dried on filter paper for 24 hours at room temperature (in the shade).

Table 1: The experiment design of Citrus seeds.

Control	Seed nested with distilled water for three hour
Experiment	Seed treated with hormones for three hour

After hormone treatment
For germination, all seeds were sterilized in a 5 percent sodium hypochlorite solution for three minutes and then sown in different pots. Control pot, 10 ppm, 30 ppm, 50 ppm auxin pot, 10 ppm, 30 ppm Cytokinin pot, and 1:1, 1:2, and 2:1 ppm pot, respectively. Next, 0.5 ml Distilled water was poured into the little control pot, and Harmons, respectively, 0.5 ml 10 ppm, 30 ppm, 50 ppm auxin and 0.5 ml 10 ppm, 30 ppm, 50 ppm Cytokinin and 0.5 ml 1:1, 1:2, and 2:1 ratio of auxin and Cytokinin, were poured into the experimental pot. All pots were exposed to sunlight for one hour after adding distilled water and hormone. After one hour, add about water to compensate for the soil's moisture. This procedure was repeated every day until germination was visible. Measure the shoot and root sire after germination.

Results and Discussion
The processes of seed germination have a significant impact on crop yield. A variety of elements, including plant hormones, influence these processes. Plant hormones, which are produced by both plants and microbes in the soil, can have a big impact on seed germination. The observations of the experiments as shown as follows.

Results of Control
Lemon seed germination was monitored daily for 31 days, with a root size of 3.1 cm and a germinated shoot size of 3.7 cm in the control group. (Table 2).

Table 2: Seed germination in control after 31 days

Control	Root Size (cm)	Shoot size (cm)
Distilled Water	3.1	3.7

Results of Auxin
After 31 days, records showed 3.3cm root size and 3.9cm shoot size at 10 ppm auxin, 3.8 cm root size and 3.2 cm shoot size at 30 ppm auxin, and 4.0 cm root size and 3.5cm shoot size at 50 ppm auxin (Table 3). It signifies that a 10 ppm dose produces positive outcomes for shoot elongation and a 50 ppm dose produces significant root elongation (Fig.1). Researchers that looked into development and growth, as well as the formation of vascular tissue, came up with comparable findings [35, 36].

Table 3: Effect of Auxin on Seed germination of lemon (After 31 Days)

Auxin	Root size (cm)	Shoot size (cm)
10 ppm	3.3	3.9
30 ppm	3.8	3.2
50 ppm	4.0	3.5

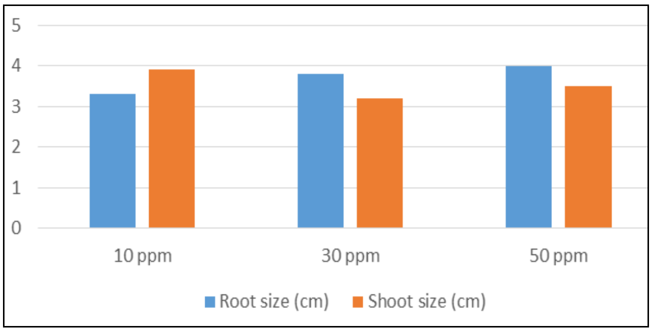

Fig 1: Effect of Auxin on Seed germination of lemon (After 31 Days)

Results of Cytokinin
After 31 days, the root size was 2.9 cm and the shoot size was 4.4 cm at 10 ppm, 3.2 cm root and 4.0 cm shoot size at 30 ppm, and 3.1 cm root and 3.9 cm shoot size at 50 ppm. Cytokinin (Table 4). In 10 ppm Cytokine, there is a very slight increase in shoot size when compared to control, while 10 ppm followed by 30 ppm Cytokine produced excellent results (Fig. 2).

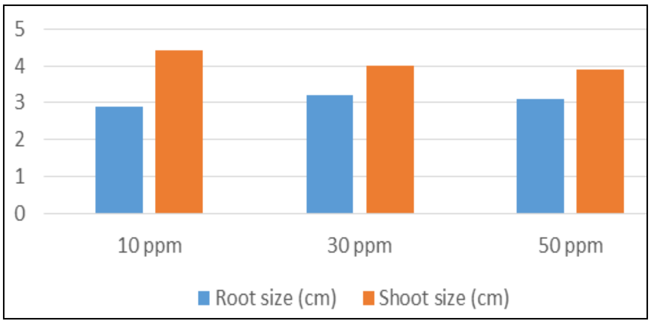

Fig 2: Effect of Cytokinin on Seed germination of lemon (After 31 Days)

Table 4: Effect of Cytokinin on Seed germination of lemon (After 31 Days)

Cytokinin	Root size (cm)	Shoot size (cm)
10 ppm	2.9	4.4
30 ppm	3.2	4.0
50 ppm	3.1	3.9

Results of ratio of Auxin-Cytokinin
After 31 days showing 3.4 cm root size and 4.9 cm shoot size at 10 ppm, 3.6 cm root size and 5.6 cm shoot size at 30 ppm, 3.7 cm root size and 4.5 cm shoot size at 50 ppm auxin cytokinin ratio (Table 5).

Positive results were obtained in both root and shoot growth when compared to the control. The root grows the most at 50 ppm, followed by 30 ppm, and the shoot grows the most at 30 ppm. This is the maximum height and positive experimental observations based on all of the above observations (Fig. 3).

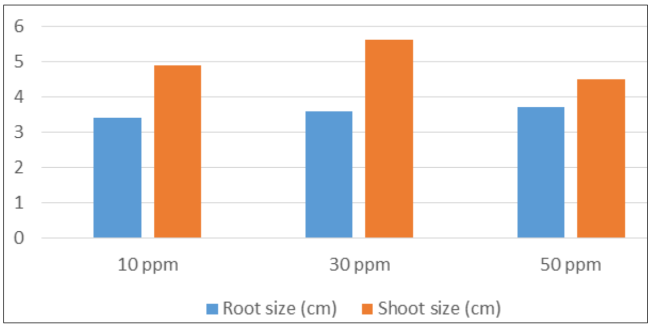

Fig 3: Effect of ratio of auxin and Cytokinin on seed germination of lemon (After 31 Days)

Table 5: Effect of ratio of auxin and Cytokinin on seed germination of lemon (After 31 Days)

Auxin: Cytokinin	Root size (cm)	Shoot size (cm)
10 ppm	3.4	4.9
30 ppm	3.6	5.6
50 ppm	3.7	4.5

Summary
The processes of seed germination and dormancy have a significant impact on crop yield a variety of elements, including plant hormones, influence these processes. Plant hormones, which are produced by plants as well as microbes in the soil, can considerably impact seed germination. Plant hormones such as ethylene, gibberellins, cytokinins, and IAA can affect seed germination positively or negatively, depending on how they interact with one another. Plant genes and hormones interact in a variety of ways. Plant hormones activate some plant genes required for the function of plant hormones and other plant genes. The molecular pathways for plant hormone perception identified by proteomic and molecular biology investigations may reveal further insights into the impact of plant hormones on seed germination. Other fresh and exciting discoveries about seed biology and behaviour under various settings have also been made. By performing this work, we show that when the increasing concentration of auxin (ppm), root size also increased at that time and variation seen in shoot size. So auxin is more affected on root size compare to shoot size. When an increase in Cytokinin (ppm) concentration, shoot size decreases at that time and variation seen in root size; Cytokinin is more affected by shoot size than root size. In a different ratio of auxin and Cytokinin, root size and shoot size is different. While using auxin cytokinin ratio positive results were obtained in both root and shoot growth when compared to the control. The root grows the most at 50 ppm, followed by 30 ppm, and the shoot grows the most at 30 ppm. This is the maximum height and positive experimental observations based on all of the above observations. Future research should focus on how a combination of acceptable agricultural practices and biological methods, such as the utilization of soil bacteria, can offer a suitable environment for seed germination and growth in various situations. More information about hormonal signalling during seed germination and seed biology is still needed. For example, seed germination may be regulated during dormancy and how seed germination speed can be increased by altering seed behaviour under various situations.

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Conflict of interest
The authors declare no conflict of interest

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