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Research Article
Effects of Phytohormone on Seed Germination, Seedling Vigour and the Phytochemical Contents of Three Cucurbits

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Abstract

Background and Objective: A comparative study was carried out on the effect of phytohormone on seed germination, seedling vigour and the phytochemical content of three cucurbits, which are *Cucumis melo* (L.), *Lagenaria breviflora* (Benth) and *Citrullus lanatus* (Thunb). Phytochemical analysis of air-dried, powdered epicarp, mesocarp and seeds of the mature fruits were carried out, also the effects of varying concentrations of indole acetic acid (IAA), naphthalene acetic acid (NAA) and gibberellic acid (GA_3) on germination and seedling vigour of these 3 cucurbits were investigated. The study is designed to obtain the inhibitory and the stimulatory effects of the 3 cucurbits used in this research. **Materials and Methods:** Treatments were arranged in 5 replicates and monitored for 16 days. Experiments were carried out in 9 cm petri dishes in the laboratory. Data were subjected to two-way analysis of variance (ANOVA) at $p<0.05$. Means were compared using LSD. **Results:** The study revealed that the mean percentage germination and seedling vigour of *Cucumis melo* (*C. melo*) was significantly higher ($p<0.05$) in the control than in 100-500 ppm concentrations of GA_3, IAA and NAA. Phenol was not detected in the epicarp and mesocarp of *C. lanatus* and seeds of *L. breviflora*. However, concentrations of phenol detected in the different parts of *C. melo* and *Lagenaria breviflora* (*L. breviflora*) (epicarp, mesocarp and seed) were not significantly different. The concentrations of the phytochemicals were significantly ($p<0.05$) different among the epicarp, mesocarp and seeds of the cucurbits except for alkaloid. However, concentrations of phenol detected in the different parts of *C. melo* and *L. breviflora* were not significantly different ($p>0.05$). Tannin was not detected in the epicarp, mesocarp and seeds of the three cucurbits. Flavonoid was also significantly higher ($p<0.05$) in the epicarp of *C. melo* and *L. breviflora* than in their mesocarp and seeds. **Conclusion:** Conclusively, effects of varying concentrations of IAA, NAA and GA_3 was not significant on the parameters studied. Phytochemicals were detected in the epicarp, mesocarp and seeds of the cucurbits under investigation. This study revealed the phytochemical contents and the effects of hormones on the germination of seeds including the vitality of the seed produced.

Key words: Seed germination, seed vigour, varying concentration, comparative, phytochemical, seeds, fruits

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cucurbitaceae comprises both cultivated and wild species. Many of these have been domesticated and grown as vegetables forming basic ingredients for human diet[1]. They are herbaceous vines[2], commonly called the "gourd family" of flowering plants and collectively known as cucurbits[3], they contain about 125 genera and 980 species and are well represented in Nigeria by 21 genera, many of which are considered economically important[4]. They are cultivated in different parts of the world for their medicinal value, this value is as a result of certain biological active substance called phytochemicals that are found in them.

Cucurbitaceae are herbaceous vines divided into two subfamilies: *Zanonioidae* and *Cucurbitoideae*, they are commonly called the "gourd family" of flowering plants and collectively known as cucurbits[5]. They contain about 125 genera and 980 species[6]. Phytochemicals are bioactive, non-essential plant nutrient chemical compounds also called phytonutrient. They are rich potential source of drugs as they produce a vast array of novel bioactive molecules many of which probably serve as chemical defenses against infection, making them useful therapeutically.

Phytohormones or plant growth regulators (PGRs) are endogenous substances (chemical) synthesized by plant for the promotion or inhibition of some metabolic processes in plants. In small amounts they modify the natural growth regulatory systems of the plant right from seed germination (i.e., it regulates physiological processes), a quick means of increasing crop production. They can be natural or synthetic however, they play a central role in morphology and physiology of plants. The major target of this research work is to know the effects of the aforementioned phytohormones on the growth, germination and vitality on the curcubites species to be used, however, the application of this hormone varies in their effects and this research is geared towards obtaining the quantity of application of these hormones to achieve quality results.

MATERIALS AND METHODS

Evaluation of Phytochemical constituents of *Lagenaria breviflora, Cucumis melo* and *Citrullus lanatus* fruit parts.

This was carried out using the method described by Ciulci[7]. The seeds, mesocarp and epicarp of the fruits were screened for the following phytochemicals including the alkaloids, saponins, tannins, flavonoids, glycosides, phylobatannins, phenol and anthraquinone.

Preparation of seeds for germination: Air dried seeds were prepared for germination according to the method of Agboola and Adedire[8]. These petri dishes were washed with sterile distilled water, rinsed and surface sterilized using ethanol and cotton wool. Two filter papers of 9 cm were put into the petri dishes.

Germination studies: Viability of the seeds was determined via floatation method. Seeds were prepared for germination according to the method of Etejere *et al.*[9]. Seeds were surfaced sterilized with 5% solution of hypochlorite solution for 5 min and then washed severally in distilled water. Twenty seeds were plated in the Petri dishes and moistened with 2.5 mL distilled water. Five replicates of the set up were made.

Preparation of varying concentrations of IAA, NAA and GA_3: Preparation and exogenous application of some phytohormones were carried out according to the method described by Sajid *et al.*[10].

Gibberellic acid (GA_3): Approximately 0.15 g of GA_3 was dissolved in 5 mL of organic solvent (ethanol). It was then made up to 300 mL with distilled water to give 500 mg L^{-1} stock solution. From the stock, 100, 200, 300, 400 and 500 ppm of GA_3, indole acetic acid (IAA) solution was prepared. IAA (0.16 g) was dissolved in 5 mL organic solvent (ethanol) and made up to 200 mL with distilled water to give 800 mg L^{-1} stock solution. 100, 200, 300, 400 and 500 ppm were then prepared from the stock solution.

Naphthalene acetic acid (NAA): Approximately 0.15 g of NAA was dissolved in 5 mL of organic solvent (ethanol). It was then made up to 300 mL by adding distilled water to give 500 mg L^{-1} stock solution. From the stock, 100, 200, 300, 400 and 500 ppm of NAA solution was prepared.

Seeds from each sample were then soaked in each of the prepared solutions of GA (100-500 ppm), IAA (100-500 ppm) and NAA (100-500 ppm) for 13 h and were plated for germination test.

The experimental design was $3\times3\times5$ factorial with 3 Curcubit types, 3 plant hormones and 5 concentrations in a randomized design.

Fruit and seed collection: Mature fruits of the three members of the *Cucurbitaceae* family (*Lagenaria breviflora*, *Cucumis melo*, *Citrullus lanatus*) were randomly selected and

bought from local farmers at Osiele market in Abeokuta, Ogun State. The seeds were extracted manually from their mature fruits which were left for 10 days to rotten in the case of *Lagenaria breviflora* and *Cucumis melo.* The seeds of *Citrullus lanatus* were also extracted manually after the mature fruit have been cut longitudinally with a knife. Ten g of the seeds was grounded using a sony electric blender into powdery form which was used for proximate and phytochemical analysis.

Each longitudinally dissected mature fresh fruit was peeled to collect their epicarp and mesocarp which were oven dried and ground seperately to powdery form for proximate and phytochemical analysis.

Preparation of extracts: Extracts were prepared from epicarp, mesocarp and seeds of the three *Cucurbitaceae* (*Lagenaria breviflora*, *Cucumis melo* and *Citrullus lanatus*) using the method described by Dangeti *et al.*[11].

Statistical analysis: All data collected from the above experiments were analyzed using two-way analysis of variance (ANOVA) and means were compared using LSD. p-value was set at $p \leq 0.05$. as discussed by Schlotzhauer and Littell[12].

RESULTS

The percentage concentration of some phytochemicals detected in the epicarp, mesocarp and seed of *C. melo, L. breviflora* and *C. lanatus* is shown in Table 1.

The concentrations of the phytochemicals were significantly ($p<0.05$) different among the epicarp, mesocarp and seeds of *C. melo, L. breviflora* and *C. lanatus* except for alkaloid, phenol and anthraquinone present in *L. breviflora* (epicarp, mesocarp and seed) which were not significantly ($p>0.05$) different.

Phenol was not detected in the epicarp and mesocarp of *C. lanatus* and seeds of *L. breviflora*. However, concentrations of phenol detected in the different parts of *C. melo* and *L. breviflora* (epicarp, mesocarp and seed) were not significantly different ($p>0.05$) as shown in Table 1.

Table 1 also shows that tannin was not detected in the epicarp, mesocarp and seeds of *C. lanatus* and in the seeds of *C. melo* and *L. breviflora.* Flavonoid was also significantly higher ($p<0.05$) in the epicarp of *C. melo* and *L. breviflora* than in their mesocarp and seeds.

Table 2 shows the percentage germination and seedling vigour were significantly higher (63%) in *C. melo* treated with 400 ppm but not significantly different from those treated with 300 ppm GA_3 compared with those treated with 100, 200 and 500 ppm. However, no significant difference ($p>0.05$) was observed in the percentage germination and seedling vigour of *C. melo* treated with varying concentrations (100, 200, 300, 400 and 500 ppm) of IAA and NAA.

No significant difference ($p>0.05$) was recorded in the values of percentage germination and seedling vigour of *L. breviflora* seeds treated with different concentrations of GA_3 (100, 200, 300, 400 and 500 ppm), though the seeds of *L. breviflora* treated with 200 ppm GA_3 recorded the highest percentage germination and seedling vigour as shown in Table 2.

The percentage germination and seedling vigour of *L. breviflora* seeds treated with IAA was significantly higher ($p<0.05$) particularly those treated with 100 ppm IAA. There was no significant difference ($p>0.05$) in the germination and seedling vigour of seeds of *L. breviflora* treated with varying concentrations of IAA.

Percentage germination and seedling vigour in *L. breviflora* treated with the varying concentrations of NAA were not significantly different ($p>0.05$).

Percentage germination and seedling vigour of *C. lanatus* treated with varying concentrations of GA_3, IAA and NAA were not significantly different ($p>0.05$) but, percentage germination and seedling vigour were higher in *C. lanatus* seeds treated with 200 ppm GA_3, 100 ppm IAA and 500 ppm NAA.

Average percentage germination and seedling vigour was significantly high ($p<0.05$) in the seeds of *C. melo* and *L. breviflora* plated with water (control) than those plated with varying concentrations of GA3, IAA and NAA. However, average percentage germination and seedling vigour recorded for the seeds of *C. lanatus* was highest in IAA. Comparing the percentage germination and seedling vigour of *C. melo, L. breviflora* and *C. lanatus* with varying concentrations of the hormones, 200 ppm GA_3 had the best percentage germination and seedling vigour for *L. breviflora* and *C. lanatus*. On the other hand, 100 ppm IAA resulted in the best percentage germination and seedling vigour of *C. melo, L. breviflora* and *C. lanatus*. In addition, *C. lanatus* was also best in 400 ppm IAA. No germination and seedling vigour was observed in *C. melo* seeds treated with 300 and 400 ppm NAA. However, the best percentage germination of *C. melo* was observed in 400 ppm GA_3 while the highest percentage germination of *L. breviflora* and *C. lanatus* was recorded in 100 ppm IAA (Table 2).

Table 1: Concentrations of some phytochemicals in seeds, epicarp and mesocarp of *C. melo L. breviflora* and *C. lanatus*

Phytochemicals (%)	Epicarp			Mesocarp			Seed		
	C. melo	*L. breviflora*	*C. lanatus*	*C. melo*	*L. breviflora*	*C. lanatus*	*C. melo*	*L. breviflora*	*C. lanatus*
Tannin	0.16±0.00^{c}	0.23±0.01^{c}	ND	0.95±0.07^{b}	0.17±0.01^{b}	ND	ND	ND	ND
Saponin	2.47±0.05^{c}	2.64±0.03^{c}	2.07±0.03^{c}	2.19±0.08^{b}	2.15±0.04^{b}	1.49±0.04^{b}	0.21±0.00^{a}	1.23±0.01^{a}	0.27±0.02^{a}
Alkaloid	2.04±0.06^{c}	12.43±10.02^{a}	1.87±0.01^{c}	0.95±0.01^{b}	1.25±0.02^{a}	1.13±0.01^{b}	0.42±0.11^{a}	0.54±0.03^{a}	0.45±0.01^{a}
Flavonoid	1.84±0.02^{c}	2.19±0.08^{c}	1.74±0.07^{c}	1.56±0.01^{b}	1.74±0.02^{b}	1.43±0.01^{b}	0.82±0.04^{a}	0.93±0.01^{a}	0.82±0.01^{a}
Glycoside	0.82±0.01^{c}	0.92±0.01^{c}	0.63±0.01^{c}	0.52±0.01^{b}	0.69±0.03^{b}	0.31±0.01	0.09±0.00^{a}	0.13±0.00^{a}	0.13±0.02^{a}
Phenol	0.11±0.02^{a}	0.06±0.06^{a}	ND	0.05±0.05^{a}	0.09±0.01^{a}	ND	0.06±0.06^{a}	ND	0.07±0.07^{a}
Anthraquinone	1.22±0.02^{c}	0.89±0.53^{a}	1.11±0.01^{c}	1.09±0.03^{b}	0.87±0.01^{a}	0.70±0.02^{b}	0.63±0.010^{a}	0.58±0.01^{a}	0.23±0.02^{a}
Phlobatanin	0.41±0.01^{c}	0.55±0.01^{c}	0.23±0.02^{c}	0.21±0.01^{b}	0.43±0.01^{b}	0.17±0.01^{b}	0.10±0.010^{a}	0.21±0.01^{a}	0.05±0.01^{a}

^{abc}Mean values (±Standard Error) with the same superscript in the same row for each species are not significantly different (p > 0.05) (ND = Not Detected)

Table 2: Effect of GA_3, IAA and NAA on % germination and seedling vigour of *C. melo, L. breviflora* and *C. lanatus* seeds

Hormones	Concentration (ppm)	Germination (%)			Seedling vigour		
		C. melo	*L. breviflora*	*C. lanatus*	*C. melo*	*L. breviflora*	*C. lanatus*
H_2O (Control)	0	80.0±3.87	27.00±3.00	12.00±2.55	7.42±0.28	2.26±0.24	1.18±0.26
GA_3	100	5.0±2.74^{a}	27.00±6.04^{ab}	20.00±5.24^{a}	0.48±0.24^{a}	2.50±0.54^{a}	1.72±0.46^{a}
	200	5.0±3.87^{a}	30.00±2.74^{b}	24.00±4.85^{a}	0.46±0.37^{a}	2.60±0.25^{a}	2.18±0.44^{a}
	300	54.0±4.85^{b}	21.00±1.87^{ab}	20.00±5.70^{a}	5.18±0.60^{b}	2.02±0.195^{a}	1.76±0.50^{a}
	400	63.0±10.07^{b}	23.00±4.64^{ab}	20.00±6.12^{a}	6.92±1.10^{b}	2.32±0.43^{a}	1.84±0.56^{a}
	500	6.0±2.92^{a}	16.00±4.85^{a}	21.00±5.34^{a}	0.72±0.33^{a}	1.60±0.52^{a}	2.00±0.49^{a}
IAA	100	10.0±6.52^{a}	35.00±4.18^{b}	51.00±3.32^{c}	0.72±0.45^{a}	3.18±0.35^{a}	3.88±0.29^{b}
	200	8.0±4.36^{a}	19.00±4.30^{a}	44.00±4.30^{bc}	0.68±0.35^{a}	1.62±0.35^{a}	3.66±0.38^{b}
	300	4.0±2.92^{a}	22.00±2.55^{a}	31.00±3.32^{ab}	0.44±0.33^{a}	1.94±0.23^{a}	2.64±0.29^{ab}
	400	9.0±2.92^{a}	17.00±1.22^{a}	34.00±6.96^{ab}	0.98±0.33^{a}	1.40±0.15^{a}	2.94±0.70^{ab}
	500	6.0±2.92^{a}	24.00±2.92^{a}	24.00±4.30^{a}	0.72±0.33^{a}	1.98±0.27^{a}	1.96±0.34^{a}
NAA	100	2.0±2.0^{a}	6.00±2.45^{a}	5.00±1.58^{a}	0.12±0.12^{a}	0.36±0.15^{a}	0.30±0.95^{a}
	200	3.0±1.23^{a}	4.00±1.87^{a}	6.00±2.92^{a}	0.20±0.08^{a}	0.20±0.09^{a}	0.34±0.16^{a}
	300	0	3.00±2.00^{a}	8.00±2.00^{a}	0	0.16±0.10^{a}	0.46±0.12^{a}
	400	0	1.00±1.00^{a}	6.00±1.87^{a}	0	0.06±0.06^{a}	0.34±0.10^{a}
	500	1.0±1.0^{a}	7.00±2.55^{a}	8.00±3.39^{a}	0.06±0.06^{a}	0.34±0.12^{a}	0.48±0.20^{a}

^{abc}Mean values (±Standard Error) with the same superscript in the same row for each species are not significantly different (p>0.05), GA_3: Gibberellic acid , IAA: Indole acetic acid and NAA: Naphthalene acetic acid

DISCUSSION

Phytochemicals of medicinal importance such as glycosides, tannin, alkaloids, flavonoids, anthraquinone, phenol, saponins and phlobatanins were detected in the epicarp, mesocarp and seed of *C. melo, L. breviflora* and *C. lanatus*. Parivuguna *et al.*[13], reported that phytochemicals and other chemical constituents are responsible for the medicinal value of plants. The presence of these phytochemical constituents in fruits has also been reported to account for the counter reaction observed against metallic taste due to chemotherapy noticed in the mouth of cancer patients[14]. The occurrence of these phytochemical in the fruit parts utilized in this study conforms to the report of Joshi *et al.*[15] and observed that cucurbits are known to contain these bioactive compounds which aids colour, flavour and generally play protective roles in these fruits.

This study recorded high saponin concentrations in the epicarp of the fruits of *C. melo, L. breviflora* and *C. lanatus.* Saponin is said to have hypotensive and cardiodepressant properties[16]. Recent studies have shown that saponins possess haemolytic and induced cytotoxicity effect[17], anti-tumor and anti-mutagenic properties and can reduce the risk of cancer by preventing the growth of the cancer cells[18]. Cucurbits plants as *L. breviflora* are used in the treatment of wounds and also to stops bleeding[19]. Saponin exhibit foaming or soapy properties and cell membrane permeabilizing properties, this soapy character is due to their surfactant properties[20].

This study revealed levels of glycosides in the epicarp, mesocarp and seed of *C. melo, L. breviflora* and *C. lanatus*. Glycosides have anti-inflammatory effects, protecting against lethal endotoxemia[21] and are useful in cardiac treatment of congestive heart failure and cardiac arrhythmia[22].

C. melo and *L. breviflora* contained some percentage of tannin repoted by Njoku and Akumefula[23] that tannin has astringent properties, (causes the dry and puckery feeling felt in the mouth following consumption of unripe fruit) and hasten wound healing[24]. Tannins are potential metal chelators, precipitators and biological antioxidants, they usually form insoluble complexes with protein, interfering with their bioavailability[25]. Tannins have been reported in the fruits of other cucurbits as *Cucumis sativa* and *Praecitrullus fistulosus*[26]. Their high content in diets leads to poor palatability.

Tannin was not detected in *C. lanatus* probably due to loss of its astringency to fruit ripening since the destruction and modification of tannins overtime plays an important role in ripening of fruits[27].

Flavonoids are known to aid colour and flavour in plant, its content in the epicarp of the fruits parts in this study was high probably accounting for the attractive colouration of the epicarp of these fruits. The content of Flavonoid in the epicarp of *C. melo* and *L. breviflora* was significantly high ($p<0.05$), the same was also recorded in the seeds of *C. lanatus*.

Flavonoids are anti-microbial and inhibitory in nature, this attribute is ernested in preventing the initiation and promotion of tumors[28]. Flavonoids help in stress reduction by scavenging hydroxyl and lipid peroxy radicals, superoxide and anions and has been recognized as an anticoagulant and an aphrodisiac[29].

In contrast, phenol is known to promote the production of undesirable colour, flavour and also loss of nutrient, their content in the fruit parts studied here were very low, this probably giving these fruit an edge nutritionally.

Alkaloids was present in the fruit parts of *C. melo, L. breviflora* and *C. lanatus* examined in this study. Alkaloids are generally known to be highly toxic made up of ammonium compounds, they defend plants against herbivores and pathogens[30]. This property is ernested and used in the reduction and elimination of cancer cells, also applied as anesthetics and CNS stimulants[31]. Due to its toxicity, it is normally applied at a strictly controlled dose in herbal medicine[32].

The use of phytohormone is becoming increasingly important in agricultural and horticultural practices for many cultivated plants[33]. Its use in pre-sowing seed treatment plays an important role in regulating germination and vigour[34].

The hormones, GA_3, IAA and NAA used at various concentrations 100, 200, 300, 400 and 500 ppm exerted the same effect on the percentage germination and seedling vigour of *C. melo, L. breviflora* and *C. lanatus* seeds.

GA_3 greatly enhanced the percentage germination and seedling vigour in *C. melo* seeds at 300 and 400 ppm. This may be attributed to the fact that at a high concentration the stimulation of the expression of enzymes increased affecting both the physiological and metabolical activities within the seeds[35] there by, promoting embryo growth and reducing the physical restraint imposed by the endosperm or seed testa making way for the protrusion of the radicle. Also, GA_3 at higher concentrations is effective in overcoming dormancy thereby, causing rapid germination of seeds[36].

The above result conforms with the report of Ebofin *et al.*[37], Joshi *et al.*[15] and Singh and Murthy[38]. At higher concentrations they observed increased germination working with *Cassia obtusifolia*, Savanna tree legumes and *Pyracantha crenulata*, respectively. This is in variance with Gunasekran *et al.*[17] and Zahedi *et al.*[34], who, respectively

reported on *Dianthus barbatus* and *Myristica fragrans*, at a low concentration of 100 ppm GA_3, these seeds had very high germinability.

In addition[13], 100% germination in *S. glauca* using GA_3 pre-treatment at 150 and 200 ppm, conformed with the result recorded for *L. breviflora* and *C. lanatus*, at 200 ppm GA_3 in this study.

The effect of IAA on the percentage germination and seedling vigour was greatly enhanced. With *C. Lanatus* and *L. breviflora* responding more positively at 100 ppm. This is probably due to the fact that IAA exerts its stimulatory effect on germination and plant growth within a narrow concentration range, beyond which the seed may become unresponsive and growth inhibited[39], also the inherent characteristic of IAA in improving water content, protein synthesis of the seed at lower concentrations which promotes cell elongation and different iation increasingly pocotyllength[40].

In this study NAA was not very effective. The percentage germination and seedling vigour at 16 days was generally low at all the concentrations for *C. melo, L. breviflora* and *C. lanatus*, particularly at 300 and 400 ppm where zero percentage germination and seedling vigour at 16 days was recorded for *C. melo* seeds. This may be due to the fact that these concentrations utilized in this study were still too high for NAA to exert much effect on the seeds. Shinde *et al*.[36] observed that the exogenous application of naphthalene acetic acid (NAA) at low very concentration increased percentage germination and seedling vigour.

Furthermore, the average percentage germination and seedling vigour were found to be significantly higher ($p<0.05$) in *C. melo* and *L. breviflora* seeds samples plated with water (control) than those plated with the different hormone GA_3, IAA and NAA.

The various concentrations of hormone, GA_3, IAA and NAA used had the same effect on the plumule and radicle length of *C. melo, L. breviflora* and *C. lanatus*. Four hundred ppm GA_3 recorded the highest plumule and radicle length in *C. melo* and *C. lanatus.*

CONCLUSION

This study has been able to show the phytochemical contents and the effects of hormones on the germination of seeds including the vitality of the seed produced. According to the study, presence of high percentage of hormones such as giberellins affects positively the germination ability of the seeds used, especially when added before planting. These effects were majorly observed in the growth parameters which include the radicle and plumule lengths. In contrast, addition of naphtalene at high concentration reduces the germination ability of the species used. However, these effects must be taken into cognicance for optimal yield.

SIGNIFICANCE STATEMENT

This study discovers the phytochemical content of seeds and fruits of the three cucurbits used in this study, it also helps in getting the phytochemical characteristics of the species, obtaining improved seed vigour, increasing seed germination and also in understanding the varying concentration of hormones in seeds. This study will help the researcher to detect important areas of the physiology of seeds and seed germination which include observing phytohormones response on the aforementioned. More importantly, caution and necessary pre-germination tests may be revealed from this study.

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