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Direct Organogenesis Of Hypocotyl Explants From *In Vitro* Seedlings Of *Cucumis Anguria* L.

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Abstract
The Plant growth regulator BAP along with KIN, 2, 4 – D and IBA combination of hormones 1.0 + 0.5 to 4.0 + 2.0 mg/l induced shoots ranging from direct organogenesis of hypocotyl explants from *in vitro* seedlings of *Cucumis anguria* L. The length of the shoot ranges from 3.62 to 4.70 cm. When BAP along with IBA was tested for shoot initiation and elongation, remarkable results were observed with a mean shoot length of 7.2 cm. In this experiment it was found that BAP with IBA was suitable for multiple shoot initiation and elongation of shoots with direct organogenesis of hypocotyl explants of *C. anguria* .

Introduction
The Cucurbitaceae have an important cultural and economic role among many societies. Some species are among the plants first domesticated by humans and several are stable crops (Jeffrey, 1990). West Indian gherkin (*Cucumis anguria* L.) is a highly nutrient vegetable with traditional medicinal value, mainly cultivated and consumed in Africa, Brazil, Cuba, India, United States and Zimbabwe (Mangan *et al.*, 2010). It belongs to the subgenus *Melo*, and is used in a similar fashion as of cucumber (Kirkbride, 1993). The gherkin (*Cucumis anguria* L.) is an important horticultural crop, mainly cultivated and consumed in Africa, Brazil, Cuba, India, United States and Zimbabwe. The fruits of gherkin are consumed as boiled, fried, stewed, pickled, and fresh in salads and also in hamburgers.

The fruit of the vegetable contain high amounts of protein, calcium, phosphorous, iron and vitamin C (Whitaker and Davis, 1962). The gherkin is also known for traditional importance in medicinal to treat stomach ache, jaundice, hemorrhoids and preventing stone formation in kidney (Baird and Thieret, 1988; Schultes, 1990). The West Indian Gherkins are resistant to Cucumber Green Mottle Virus (CGMV) (Kroon et al., 1979; Visser and Dennijs, 1983), Zucchini Lethal Chlorosis Virus (ZLCV) (Giampan et al., 2007). Fungal disease *Fusarium* wilt (*Fusarium oxysporum*) (Thomas and More, 1990), and Powdery mildew (Lebeda, 1984). However, this plant is seriously affected by leaf spot disease and caused more than 75% crop damage. The *in vitro* multiple shoot formation and subsequent root induction considering various cultural aspects using nodal explants of *Cucumis anguria* L. derived achieved on MS medium containing BAP (1mg/l), NAA (0.2mg/l) and L - glutamine (20mg/l). The *in vitro* multiple shoot formation and subsequent root induction considering various cultural aspects using shoot tip explants of *Cucumis anguria* L. derived on MS fortified with 2 mg/l 6-benzyl amino purine (BAP) and 0.5 mg/l indole acetic acid (IAA) (Senthil kumar, 2013). The *in vitro* clonal propagation studies on *Trichosanthes cucumerina* showed responses on multiple root induction by using the nodal and shoot tip explants (Devendra *et al*., 2008). We also evaluated its survival percentage in field conditions during summer (March - June, 36–42 °C), spring (July – October, 28–35 °C) and winter (November - February, 24–27 °C) seasons. Regeneration via indirect organogenesis will also be useful for genetic transformation studies and production of somaclonal variants in *C. anguria*, further this robust protocol may also be applicable to other cucurbits.

Materials and Methods
Source of seeds
The collected seeds were grown in the College Botanical garden of Jamal Mohamed college, Tiruchirappalli,Tamil Nadu, India, *in vitro* germinated plants as source of explants.

Sterilization of seeds
Mature seeds of West Indian Gherkin (*Cucumis anguria* L.) were used as explant source. The seeds were surface sterilized by washing in teepol solution (Reckitt Benckiser Ltd., Kolkata, India) for 15 mins and rinsed with distilled water for five times to remove the soap solution. The soaked seeds were treated with 70% ethyl alcohol for 30 seconds and then washed with sterile distilled water for three to five times. Then the seeds were finally sterilized with 0.1% (w/v) mercuric chloride solution for 3 mins. Finally the seeds were washed with sterile distilled water for five times.

The surface sterilized seeds were aseptically inoculated on MS basal medium in culture tubes (25 x 150 mm) (Borosil, India) for germination. The inoculated seeds were kept in dark for 2 days to render uniform germination and then in light for subsequent days.

Preparation of explants
Cotyledon explants were prepared by removing the seed coats with the help of a forceps and a scalpel. The two cotyledons were carefully separated eliminating the embryonic axis. The proximal half of the cotyledon (1.0 cm – 7th day old) Shoot tip and nodal explant (2.0 cm) excised from 15 day old *in vitro* raised seedlings was selected and used as explants for direct organogenesis.

Result and Discussion

Initiation of Cultures

Seeds were germinated under controlled environment with help of half strength MS basal medium from the 7-days old *in vitro* seedlings, hypocotyl explants were excised and cultured on MS medium augmented with 0.5-2.5 mg/l BAP for culture initiation.

Effect of growth regulators

In this study, the greenish shoot buds initiated and emerged from the hypocoty explants West Indian gherkin. It was represented in cultured on MS medium supplemented with individual treatment of NAA or IBA alone, the concentration ranging from 1.0 mg/l to 4.0 mg/l. About 82% of explants produced an average of 10 shoots per explant in medium containing IBA (3.0 mg/l) whereas 74% of explants produced 9 shoots per explant in medium with NAA (4.0 mg/l) only. In the present study IBA (3.0 mg/l) induced more number of shoots from nodal explants (Plate-1).The higher percentage of explants (82%) response found in NAA (4.0 mg/l) treatment. which however resulted in lower shoot production.The multiple shoots obtained in the concentrations of IBA, NAA in the initial culture with an average height of 3.23 – 5.33 cm were transferred.After sufficient elongation of shoots were transferred into rooting medium.

Regeneration on multiple shoot induction

The micro shoots formed from hypocotyl explants of *Cucumis anguria* L. Meristem culture provides a reproducible and economically viable method for producing pathogen free plants. As meristem tips are free from viruses, elimination and generation of virus free plants are possible through meristem. hypocotyl explants were placed on MS medium supplemented with different concentrations of BAP and IBA alone and along with KIN in concentrations ranging from 1.0-2.5 mg/l for BAP and IBA, 0.5 mg/l of KIN were used. The shoots were initiated within 2 weeks of culture. Among the different concentrations of IBA (2.0 mg/l) was found the best for higher frequency (96.24%) of shoot bud regeneration with maximum number of shoots (5.42±0.72 shoots/culture) compared with BAP alone were used.

The combination of BAP and IBA along with KIN has been used for multiple shoot formation; IBA with KIN was found higher response for shoot regeneration. More effective for induction of multiple shoots from shoot tip explants was found best results were recorded with IBA compared with BAP and combination with KIN. The regenerated multiple shoots were elongated in the same hormonal concentration with a maximum shoot length of 7.2cm.

Root proliferation

After 10 days of incubation, enlargement of most of the explants was observed. Direct shoot formation of shoots was observed after three weeks from mid rib region or basal petiolar region or apical region of the hypocotyls explants. Plant growth regulator BAP along with KIN, 2, 4-D and IBA combination of hormones induced shoots ranging from 1.0+0.5- 4.0+2.0 with a shoot length ranging from 3.62 - 7.2 cm. When BAP along with IBA was tested for shoot initiation and elongation there was remarkable results were observed at the concentration of 3.0+1.5 mg/l, 90% of response occurred and 19.0 mean numbers of shoots was recorded with a mean shoot length of 7.2 cm (Table). Shoot initiation percentage has been compared with other hormone concentrations like BAP with KIN and 2, 4-D was lees number of shoots per explants observed. In this experiment BAP with IBA was suitable for multiple shoot initiation and elongation of shoots with direct organogenesis of hypocotyls explants of *C. anguria.*

Conclusion

Plant growth regulator BAP along with KIN, 2, 4-D and IBA combination of hormones induced shoots ranging from 1.0 + 0.5 - 4.0 + 2.0 mg/l with a shoot length ranging from 3.62 - 4.70 cm. When BAP along with IBA was tested for shoot initiation and elongation, remarkable results were observed at the concentration of 3.0 + 1.5 mg/l, 90 % of response observed and 19.0 mean numbers of shoots were recorded with a mean shoot length of 7.2 cm. Shoot initiation percentage has been compared with other hormone concentrations like BAP with KIN and in 2, 4-D was less number of shoots. In this experiment in BAP with IBA was suitable for multiple shoot initiation and elongation of shoots with direct organogenesis of hypocotyl explants of *C. anguria* .

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Annexure

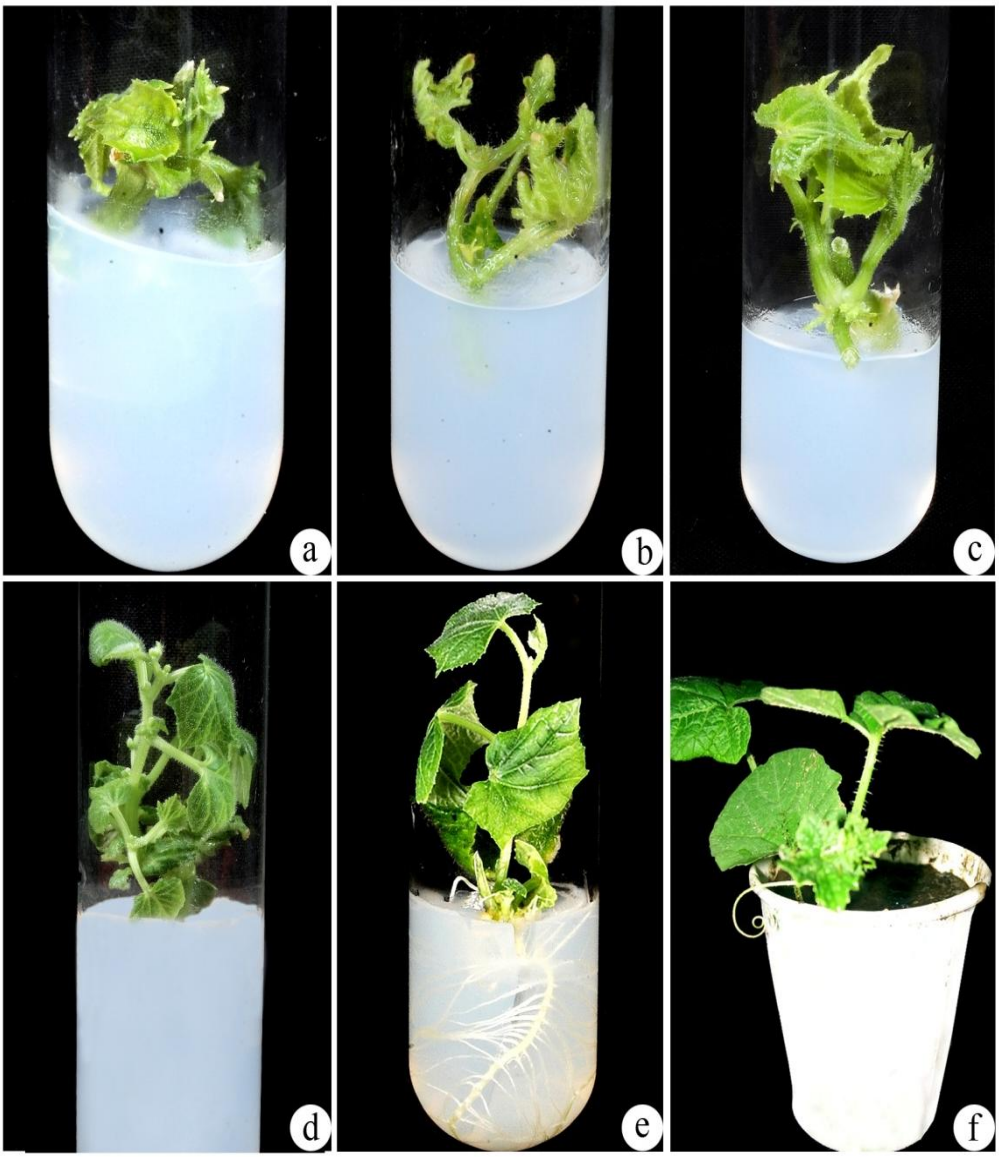
Table 1: Effect of auxin and cytokinin on shoot and root initiations from hypocotyl explants through direct organogenesis of *Cucumis anguria* L.

Plant growth regulators	Concentration (mg/l)	Regeneration Frequency (%)	Mean number of shoots	Number of roots	Mean Shoot length (cm)
BAP+2,4-D	1.0+0.5	45	10.52±0.10	4.67±0.74	3.60±0.10
	2.0+1.0	50	12.73±0.20	6.67±0.94	3.60±0.29
	3.0+1.5	63	15.97±0.12	7.00±1.63	4.70±0.25
	4.0+2.0	72	16.83±0.17	8.27±1.88	5.80±0.16
BAP+KIN	1.0+0.5	78	17.50±0.12	8.27±1.88	6.25±0.40
	2.0+1.0	82	18.00±0.29	9.00±1.00	6.50±0.00
	3.0+2.5	74	17.03±0.10	8.50±0.80	6.00±0.80
IBA+NAA	1.0+0.5	85	18.30±0.80	6.77±0.94	6.90±0.25
	2.0+1.0	75	17.93±0.25	8.00±0.08	5.90±1.80
	3.0+1.5	90	19.00±0.00	9.50±2.10	7.20±0.10

Each value represents the treatment means of five replicates
Values with the same letter within columns are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Plate - 1

Production of plantlets from *in vitro* seedling hypocotyl explants through direct organogenesis of *Cucumis anguria* L.



a) Hypocotyl explants
b) *S*hoot initiation on MS medium containing BAP (1.0 mg /l) + 2,4-D (0.5 mg /l)
c) Multiple shoot on MS medium containing BAP (2.0 mg /l + KIN (1.0 mg /l).
d) Shoot elongation on MS medium containing BAP (3.0 mg /l) + KIN (2.0 mg /l).
e) Rooting plantlet on MS medium containing IBA (3.0 mg /l) + NAA(0.5 mg /l).
f) Hardened plantlet.