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Review paper

CHRONOLOGICAL REVIEW OF MICROPROPAGATION OF CUCUMBER (*Cucumis sativus* L.) AND CUCURBITS (*Cucurbita* spp.)

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Abstract

Micropropagation is a key biotechnological method for rapid and large-scale propagation of horticultural crops. Nowadays, micropropagation plays a key role in production of disease-free plants, conservation of genetic resources and it is an essential tool in crop breeding programs. Cucumber (*Cucumis sativus* L.) and cucurbits (*Cucurbita* spp.) are economically important species that have been widely studied for in vitro regeneration. This review synthesizes research protocols reported over the past decades, focusing on explant type, culture media, and plant growth regulator combinations. A total of 29 studies on cucumber and 11 studies on cucurbits were analyzed to identify research trends over time and critical factors affecting micropropagation. Cytokinins, mainly BAP, have been consistently effective for adventitious shoot induction, while auxins such as 2,4-D promoted callusogenesis in cucumber. Optimal regeneration of cucumber often relied on cytokinin–auxin combinations, with cotyledons and hypocotyls showing high morphogenetic potential. For cucurbits, shoot tips, nodal segments, and cotyledons have been the most responsive explants where BA-based treatments have achieved superior shoot proliferation, and IBA has supported efficient rooting. Genotype-specific responses and hormonal balance are critical factors for successful micropropagation for both crops. Well-designed micropropagation protocols are essential for efficient regeneration and genetic improvement of both crops. Understanding explant-specific and genotype-dependent responses to plant growth regulators can enhance the reproducibility and scalability of in vitro systems, opening the way for advanced breeding and biotechnological applications.

Key words: *plant tissue culture, plant growth regulators, explantants, organogenesis, callusogenesis, regenerants.*

Abbreviations: MS (Murashige & Skoog medium), BAP (Benzylaminopurine), BA (6-Benzylaminopurine), KIN (Kinetin, N6-furfuryladenine), ZEA (Zeatin), 2-iP (6-(γ,γ-Dimethylallylamino) purine), IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), 2,4-D (2,4-Dichlorophenoxyacetic acid), TDZ (Thidiazuron), GA_3 (Gibberellic acid), CH (Casein hydrolysate), BM (Basal medium), PGR (Plant growth regulator).

INTRODUCTION

Challenges for micropropagation in the 21st century include reducing production costs, increasing efficiency, developing new technologies, and combining micropropagation with other propagation systems and techniques such as microcuttings, hydroponics, and aeroponics (Cardoso et al., 2018).

There are significant achievements in horticultural crops, firmly establishing plant tissue culture as the leading frontier of innovation. Currently, its application holds great promise and offers insight into the future of agricultural practices. Micropropagation of horticultural plants by the method of plant tissue culture and tissue culture in vitro has been widely used in the creation of high-quality varieties and hybrids, the creation of varieties resistant to various diseases, the creation of haploids, as

well as the stabilization and improvement of the genetic structure of species (Baria et al., 2024).

New and modern biotechnological methods and techniques allow the improvement, creation and selection of varieties that cannot be obtained with traditional breeding methods (Koleva Gudeva & Trajkova, 2015; 2018). Cucumber, which is a widely cultivated horticultural crop with significant economic value throughout the world, produces fruits rich in proteins, minerals, carbohydrates and vitamins. A number of biotic and abiotic stresses often affect cucumber production. Significant challenges include insect pests and diseases, as well as nutrient deficiencies and adverse weather conditions, such as drought or extreme temperatures. These stresses can lead to reduced yields in severe situations (Ucar et al., 2025). Cucurbits are also an important horticultural crop group, which are grown for their fruit and edible seeds. This crop group is affected by various types of diseases, especially viral diseases such as cucumber mosaic virus, zucchini yellow mosaic virus and others, which reduce yield. To produce a disease-free plant, virus elimination is a prerequisite for successful pumpkin production (Haque et al., 2010).

Traditional breeding approaches, such as classical selection and mutation selection, have served as the primary tools for improving the gene pool of cucumber and squash. In recent decades, these conventional methods have significantly contributed to the development of new varieties of these two species, with increased yield, quality, and marketability. However, despite these advances, progress in traditional breeding remains inherently slow, often requiring multiple generations to stabilize desired agronomic traits. Studies show that cucumber plants regenerated by in vitro methods can maintain the genetic fidelity of the parent plant, and meristem culture is a unique technique for producing pathogen-free plants (Ucar et al., 2025).

CHRONOLOGICAL REVIEW OF MICROPROPAGATION RESEARCH IN CUCUMBER AND CUCURBITS

Micropropagation in cucumber (*Cucumis sativus L.*)

Table 1 presents an up-to-date chronological order of 29 relevant studies by explant type, medium, plant grow regulator/s combinations and results of cucumber micropropagation (*Cucumis sativus L.*).

Handley & Chambliss (1979) used explants of axillary buds from the genotype Carolina grown in vivo. The explants were cultured on MS medium with and without hormones. Their results showed that axillary buds did not develop in cultures placed on MS medium without hormones. The highest percentage of regeneration was obtained in medium containing 0.1 ppm KIN and 0.3 μ M NAA.

Wehner & Locy (1981) subjected hypocotyl and cotyledon explants from 85 cucumber cultivars and lines to adventitious shoot and root formation in tissue culture. They used MS medium with different concentrations and combinations of BA and NAA. The hypocotyl did not respond, while from the cotyledons of a total of 85 lines that were the subject of the study, only 25 lines formed shoots and 32 lines formed roots.

Malepszy & Nadolska-Orczyk (1983) plated cucumber leaf explants on media containing different concentrations and combinations of cytokinins and auxins. Media containing 2,4,5-T produced a characteristic callus-like gel composed of single cells and multicellular aggregates.

Rajasekaran et al. (1983) developed hypocotyl explants of cucumber that produced callus were grown in MS medium with 0.5 or 1.0 μM BA and 1.5 or 5.0 μM 2, 4-D. Somatic embryos and adventitious buds were formed when the callus was transferred to medium without growth regulators.

Hisajima et al. (1989) used apical buds, the resulting shoots were continuously propagated using combinations of BAP and IBA, whereby plant regeneration was achieved by rooting individual shoots.

Gambley & Dodd (1990) written technique for the production of de novo shoots of cucumber in the presence of cytokinin using cotyledon explants. Shoots, which arose from adventitious buds, were restricted to a specific region at the base of the cotyledon. Concentrations of the cytokinins BAP, kinetin, and 2-iP were effective in inducing adventitious buds from cotyledons.

An efficient protocol for direct shoot

regeneration from cucumber leaf explants cultured on MS medium supplemented with various combinations of plant growth regulators was developed by Misra & Bhatnagar (1995). The age of the donor plant and the size of the plant were inversely proportional to the percentage of cultures that showed shoot regeneration. For elongation, the shoots were transferred to basal medium (BM) and later rooted on BM + IBA.

Kim et al. (2000) established a method of high-frequency induction of shoots using cut hypocotyl segments bearing cotyledons. Shoots were obtained from the upper cut hypocotyls of cucumber seedlings incubated on Murashige & Skoog (MS) medium containing 2.0 mg/l zeatin. Explants with long hypocotyls and 2 cotyledons produced fewer shoots. Shoots from cotyledon hypocotyl explants were removed and rooted in MS medium containing 1.0 mg/l IAA.

Seo et al. (2000) used cucumber leaf explants, which were cultured on MS medium supplemented with different concentrations of NAA and BAP. The highest regeneration efficiency was obtained on MS medium supplemented with 5.0 µM NAA + 2.5 µM BAP.

Vasudevan et al. (2001) placed seeds, which were germinated for 48 hours in the dark on sterile moist cotton. Shoot tips were used as explants and placed on MS medium with different compositions and concentrations of auxin (2, 4-D, NAA and IAA) and cytokinins (BAP and KIN). Efficient regeneration of apical buds was obtained at a concentration of 1.0 mg/l BAP. The effect of the combination of cytokinins and auxins from a single apical bud explant on MS medium + 1.0 mg/l BAP + 0.2 mg/l NAA yielded as many as 22 plants in successive subcultures.

An efficient reproductive protocol for in vitro propagation of cucumber, conducted by Ahmad & Anis (2005) was developed from a bud explant. The addition of the case in hydrolysate (CH) to the bud induction medium significantly increased the number of buds. Optimal regeneration was observed on medium containing 1.0 µM BA and 200mg/l CH. Rooting was achieved on a medium with half MS +1.0 µM NAA. The regenerants thus obtained were successfully acclimatized in a greenhouse. However, at a high concentration of BA (5.0 µM) and the same concentration of CH, callus formation with poorly defined buds was observed after three weeks of culture.

Mohammadi & Siviritepe (2007) used apical buds as initial explants, placed them on MS medium enriched with BA and NAA, individually and in combination. Proliferation rate, shoot quality and other parameters showed that the optimal treatment was obtained with the concentration of BA.

Organogenic callus induction and shoot regeneration were achieved from cotyledon explants in the study of Selvaraj et al. (2007). About 86.2% of cotyledon explants derived from 5-day-old shoots grown in vitro produced green, compact nodular organogenic callus in MS medium supplemented with NAA (2.69µM) and BA (4.44 µM) after two successive transfers at an interval of 20 days.

In the study by Ugandhar et al. (2011) in vitro plant regeneration was obtained from cotyledon and hypocotyl segments of cucumber, which were then tested using different phytohormones, individually and in combination on MS semi-solid medium supplemented with BAP, IAA and KIN. The best results were obtained with 0.5 mg/l IAA + 3.0 mg/l BAP for bud proliferation. All regenerated plants were rooted on MS medium supplemented with 1.0 mg/l IA A.

Usman et al. (2011) investigated commercial cucumber varieties for embryogenesis and plant regeneration. Maximum callus induction was observed on MS medium supplemented with 2.0 mg/l 2,4-D, 1.5 mg/l NAA and 1.5 mg/l BAP. Cotyledon explants induced callus on medium supplemented with 4.0 mg/l BAP + 0.75 mg/l NAA. Calluses induced on medium 5.0 mg/l 2,4-D gave the highest embryo formation, while calluses induced on the medium 5.0 mg/l BAP and 1.0 mg/l NAA regenerated shoots.

Kiełkowska & Havey (2012) worked on study were in vitro grown cucumber plants produced the most flowers on MS medium without plant growth regulators or with 6 µM kinetin, while BA + NAA completely inhibited flowering. Although flowers developed on both seed-derived and micropropagated plants, they were fewer, smaller, and showed meiotic abnormalities that reduced pollen viability, with the healthiest pollen found on PGR-free or kinetin-supplemented media.

Grozeva & Velkov (2014) studied the effect of different concentrations of phytohormones on callogenesis and organogenesis in two cucumber genotypes. It was found that the rate of plant regeneration depended on the genotype, the type of explant and the culture medium. Regenerants in vitro were obtained from hypocotyl explants on culture medium with

1.0 and 2.0 mg/l BA for the Gergana variety and in 1.0 and 3.0 mg/l BA. Induction of regeneration in cotyledons was determined only in the Gergana variety on culture medium supplemented with 3.0 mg/l BA and in a combination of 0.5 mg/l IAA.

Lashin & Mamdouh (2014) aimed to develop an efficient protocol for callus induction and regeneration of cucumber. Apical buds, leaves, nodal segments and internodes of cucumber were used as initial explants. They were cultured on MS medium supplemented with different concentrations of auxins and cytokinins. Optimal regeneration was obtained on MS supplemented with BA.

Sangeetha & Venkatachalam (2014) isolated shoot tips and plated them on MS medium with different concentrations of BAP and KIN. Maximum number of shoots was achieved at 1.0 mg/l BAP. Flowering was achieved on MS + 6% sucrose + 0.5 mg/l BAP. Maximum rooting rate was achieved at 1.5 mg/l IBA+0.5 mg/l KIN.

Direct in vitro plant regeneration was investigated in the study of Shukla et al. (2014) . The concentration of endogenous auxin was quantified by HPLC in young cotyledon explants, and they observed that higher auxin content promoted callus formation and inhibited direct shoot regeneration. The efficiency of regeneration depended on the age of the explant and the cultivar.

In vitro shoot propagation of cucumber was investigated by Abu-Romman et al. (2015) by placing nodal segments as initial explants on medium supplemented with cytokinins. Shoot induction was obtained with all cytokinin combinations tested.

Alam et al. (2015) investigated the development of a rapid and efficient in vitro system for cucumber propagation and regeneration, using in vitro bud explants. The addition of cytokinin stimulated bud formation from the initial explant, with BAP being more effective than KIN. Thus, bud development was effective at a concentration of 3.0 mg/l KIN. Four combinations of NAA were used for root induction, with the best results obtained on the medium with 0.5 mg/l NAA, within three weeks of cultivation.

A reliable and highly reproducible protocol has been established by Jesmin & Mian (2016) to obtain healthy and well-formed callus from cucumber explants. The source of explants were leaves, stem and cotyledons which were placed on MS medium supplemented with 0.5 mg/l BAP + 1.0 mg/l NAA. The highest percentage of callus was obtained from stem explants on a medium supplied with 1.0 mg/l BAP. The medium supplemented with 2,4-D for callus induction promoted slow growth and poor quality callus compared to those produced on medium supplemented with NAA and BAP. The callus induced on the medium containing 2,4-D was flaky and yellow in color.

In the research of Bhardway et al. (2017) four cucumber genotypes comprising gynoemous, monoecious and two parthenocarpic genotypes were used for in vitro regeneration. Cotyledon explants were transferred to eight different media with different concentrations of IAA and BAP. All media showed different responses for all genotypes. For bud initiation, superior medium was half MS supplemented with 0.5 mg/l IAA + 2.0 mg/l BAP, while for root initiation the best medium was half MS supplemented with 0.25 mg/l IAA. Four callus initiation media showed no response.

Priyanka et al. (2019) developed an efficient protocol for in vitro regeneration of cucumber plants from hypocotyl explants. Hypocotyl explants were plated on MS medium with different concentrations of BAP. The most efficient protocol for callus induction was obtained on medium supplemented with 2.0 mg/l BAP.

Miguel (2021) used four-day-old cotyledon explants from the Wisconsin 2843 line and the commercial varieties Marketer and Negrito. Four-week culture was carried out on MS-derived shoot induction medium containing 0.5 mg/l IAA and 2.5 mg/l BAP. All explants formed callus, and in two of the three cultivars, the callus extension response was not significantly affected by incubation conditions.

Sultana et al. (2021) examined different concentrations of growth regulators and three types of explants cotyledons, hypocotyl and leaves for their efficacy on callus induction potential. Callus induction was obtained on MS 2.0 mg/l 2,4-D. The callus was transferred to 2.0 mg/l BA + 0.2 mg/l NAA+1.0 mg/l KIN. Only the cotyledons placed on a medium with 2.0 mg/l BAP + 0.2 mg/l NAA +1.0 mg/l KIN regenerated buds.

Asadi (2023) cultured hypocotyl and cotyledons on MS with different concentrations and combinations of BAP and NAA. He obtained efficient callusogenesis in both genotypes.

The highest percentage of regeneration from cotyledons was achieved on MS +3.0 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.1 mg/l NAA. Regeneration from hypocotyl segments was obtained on MS +1.0 mg/l BAP + 0.2 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA.

Lapkasov et al. (2024) isolated hypocotyl, cotyledons, epicotyl and leaf fragments of two cucumber cultivars and hybrids. The explants were placed on modified Murashige & Skoog (MS) media containing various growth stimulants. The optimal medium for the production of callus tissue from cucumber cultivars was MS + 2 mg/l 2,4- D. The most successful explants were epicotyls from the cultivar Ochiai 9.

Table 1. Chronological review of micropropagation of cucumber (*Cucumis sativus* L.).

Study	Type of explant	Medium and PGR combination	Result
Handley & Chambliss (1979)	Axillary buds from genotype Carolina	MS medium with and without hormones	Highest percentage of regeneration of MS+0.1 ppm KIN + 0.3 µ M NAA.
Wehner & Locy (1981)	Hypocotyls and cotyledons from 85 varieties and lines	MS medium with 1mg/l BAP + 1 mg/l NAA	Only 28 of the tested genotypes from cotyledon explants produced shoots.
Malepszy & Nadolska-Orczyk (1983)	Cucumber leaves from three different varieties	MS with different concentrations of auxins and cytokinins	Efficient media regeneration 1.2 mg/l 2,4,5-T and 0.8 mg/l 6-BAP or 0.4 mg/l 2,4-D and 0.8 mg/l 2iP.
Rajasekaran et al. (1983)	Hypocotyl	MS with different combinations of BA, 2,4-D, NAA and CH	Callus induction was obtained on MS medium + 1.0 µ M BA + 5.0 µM 2,4-D. Hypocotyl explants placed in liquid medium with different combinations of BA, 2,4-D, NA A and CH did not yield any results.
Hisajima et al. (1989)	Shoots	MS with different combinations of NAA, BAP and KIN	Multiple shoots were produced on a medium with a combination of 0.1 ppm NAA and 1.0 ppm BAP. MS + 0.1 ppm NAA + 0.1 ppm KIN was successful for bud formation.
Gambley & Dodd (1990)	Cotyledons	MS supplemented with cytokinins	Bud formation on medium with a concentration of 4.0 mg/l BAP, KIN, N6 (2iP) adenine.
Misra & Bhatnagar (1995)	Leaves	MS with different concentrations and combinations of growth regulators and basal medium supplemented with IBA	Successful differentiation of shoots was achieved on medium with 3.0 µM BA.
Kim et al. (2000)	Hypocotyl segments with cotyledons	MS medium supplemented with 2.0 mg/l ZEA for apical bud induction MS medium supplemented with 1.0 mg/l IAA for root induction	Adventitious buds of short length (2 mm) were obtained.
Seo et al. (2000)	Cucumber leaves	MS with different concentrations of NAA and BAP	Highest regeneration efficiency of MS + 5.0 µM NAA + 2.5 µM BAP.
Vasudevan et al. (2001)	Apical buds	MS medium containing different combinations and concentrations of auxins (2,4-D; NAA and IAA) and cytokinins (BAP and KIN)	Efficient apical bud regeneration was obtained at a concentration of 1.0 mg/l BAP. The effect of a combination of cytokinins and auxins from a single apical bud explant on MS medium + 1.0 mg/l BAP + 0.2 mg/l NAA yielded as many as 22 plants in successive subcultures.

Table 1 - continued

Ahmad & Anis (2005)	Apical buds	MS medium supplemented with BA and CH	The highest regeneration efficiency was obtained at MS + 1.0 µM BA + 200 mg/l CH. Rooting was achieved at MS + 1.0 nM NAA.
Mohammadi & Siviritepe (2007)	Apical buds	MS with different combinations of BA and NAA, individually and in combination	Optimal proliferation efficiency was obtained on medium supplemented with 0.4 µM BA.
Selvaraj et al. (2007)	Cotyledons	MS containing 2.69 µM NAA and 4.44 µM BA	Adventive shoots were produced from the callus.
Ugandhar et al. (2011)	Cotyledon and hypocotyl explants (apical buds, hypocotyl and cotyledons)	Various phytohormones, individually and in combination on MS semi-solid medium, supplemented with BAP, IAA and Kn. (MS+0.5 mg/l IAA+3.0 mg/l BA)	The best results were obtained on medium supplemented with 0.5 mg/l IAA + 3.0 mg/l BAP for bud proliferation.
Usman et al. (2011)	Cotyledons	MS medium supplemented with different combinations and concentrations of 2,4-D, NAA and BAP	The explants from seed cotyledons induced callus on medium supplemented with 4.0 mg/l BAP + 0.75 mg/l NAA. Calli induced on medium 5.0 mg/l 2,4-D gave the highest embryo formation, while calluses induced on the medium 5.0 mg/l BAP and 1.0 mg/l NAA regenerated shoots.
Kielkowska & Havey (2012)	Shoots from stem fragments	MS without regulators and with growth regulators	The highest viability and germination of pollen was demonstrated on MS + 6.0 nM KIN medium.
Grozeva & Velkov (2014)	Hypocotyl and cotyledons	MS with different concentrations and combinations of BA and IAA	Efficient regeneration from hypocotyl for the variety Gergana on MS+ 2.0 mg/l BA medium.Efficient regeneration from cotyledons for the variety Gergana on MS medium + 3.0 mg/l BA + 0.5 mg/l IAA.
Lashin & Mamdouh (2014)	Buds, leaves, nodal segments, internode explants	MS with different concentrations of auxins and cytokinins	Callus formation from leaves, nodal segment of internodes on MS medium + 1.0 mg/l NAA + 1.0 mg/l BA. Callus formation from buds on MS medium + 0.5 mg/l 2,4-D and 0.5 mg/l KIN. Efficient bud regeneration on MS + 1.0 mg/l BA medium. Rooting on MS medium + 2.0 mg/l NAA.
Sangeetha & Venkatachalam (2014)	Shoot tips	MS with different concentrations of BAP and KIN	Maximum shoot number was achieved at 1.0 mg/l BAP. Flowering was achieved on MS + 6% sucrose + 0.5 mg/l BAP. Maximum rooting rate was achieved at 1.5 mg/l IBA + 0.5 mg/l KIN.
Shukla et al. (2014)	Apical buds and cotyledons	MS with different auxin concentrations	Higher auxin (TIBA) content in cotyledons quantified by HPLC was observed for callusogenesis and direct bud regeneration. The buds were transferred to MS medium + 1.0 µM BAP, and rooting was observed on basal medium supplemented with 0.3 µM IAA.

Table 1 - continued

Abu-Romman et al. (2015)	Hypocotyl	MS medium with different concentrations of cytokinins	The longest shoots were obtained on MS + 1.0 mg/l KIN medium. The lowest responses were observed on medium with BAP, ZEA and TDZ.
Alam et al. (2015)	Bud explants	MS + BAP + KIN + NAA	Four combinations of NAA were used for root induction, and the best results were obtained on the medium with 0.5 mg/l NAA, within three weeks after cultivation.
Jasmine & Mian (2016)	Leaves, stems and cotyledons.	MS medium supplemented with 0.5 mg/l BAP + 1.0 mg/l NAA	The highest percentage of callus was obtained from stem explants at a concentration of 1.0 mg/l BAP. Callus induced on the medium containing 2,4-D was flaky and yellow in color.
Bhardway et al. (2017)	Cotyledons	Eight different media with different concentrations of IAA and BAP.	The genotypes placed on the eight different media responded differently. For bud initiation, superior medium was ½ MS supplemented with 0.5 mg/l IAA + 2.0 mg/l BAP, while for root initiation the best medium turned out to be 1/2 MS supplemented with 0.25 mg/l IAA.
Priyanka et al. (2019)	Hypocotyl	MS + BAP + IAA + IBA	Efficient shoot regeneration was achieved on MS + 2.0 mg/l BAP + 0.5 mg/l IAA and 30 µM $AgNO_3$. Rooting was achieved on medium with 2.0 µM IBA.
Miguel (2021)	Cotyledons	MS with different concentrations of IAA and BAP	Shoot induction on medium MS + 0.5 mg/l IAA + 2.5 mg/l BAP.
Sultana et al. (2021)	Cotyledons, hypocotyl and leaves	MS with different concentrations of growth regulators	Callus induction at 2.0 mg/l 2,4-D. The callus was transferred to 2.0 mg/l BA + 0.2 mg/l NAA + 1.0 mg/l KIN.
Asadi (2023)	Hypocotyl and cotyledons	MS with different concentrations and combinations of BAP and NAA	Efficient callusogenesis in both genotypes. Regeneration from cotyledons on medium MS + 3.0 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.1 mg/l NAA. Regeneration from hypocotyl segments was obtained on MS medium +1.0 mg/l BAP + 0.2 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA.
Lapkasov et al. (2024)	Hypocotyl, cotyledons, epicotyl and leaf fragments, of two cucumber varieties and hybrids.	MS which contains various growth regulators	The optimal medium for the production of callus tissue from cucumber varieties was MS + 2.0 mg/l 2,4-D.

The analysis of up-to-date studies showed that cucumber micropropagation depends severely on explant selection. While apical and nodal explants are the best for direct regeneration, cotyledons are the most variable in their response. Hypocotyls and leaf explants are mostly responding with callus formation and embryogenesis. A comparison of published micropropagation studies for cucumber (*Cucumis sativus* L.) highlights that plant growth regulators play a critical role in determining the morphogenetic response (Table 2).

Table 2. Summary of plant growth regulators used in cucumber (*Cucumis sativus* L.) micropropagation in different studies, with corresponding regenerative responses.

PGR type	PGR used	Study	Main results
Cytokinins only	BAP	Misra & Bhatnagar (1995); Mohammadi & Siviritepe (2007); Abu-Romman et al. (2015); Priyanka et al. (2019); Bhardway et al. (2017); Miguel (2021)	Shoot induction, shoot elongation, callus induction depending on explant type
	KIN	Handley & Chambliss (1979); Kiełkowska & Havey (2012); Sangeetha & Venkatachalam (2014); Alam et al. (2015)	Shoot regeneration, in vitro flowering
	ZEA	Kim et al. (2000)	High-frequency adventitious shoot formation
	2-iP	Gambley & Dodd (1990)	Adventitious bud formation from cotyledons
Auxins only	IAA	Kim et al. (2000); Ugandhar et al. (2011)	Efficient rooting of regenerated shoots
	2,4-D	Rajasekaran et al. (1983); Jesmin & Mian (2016); Usman et al. (2011); Lapkasov et al. (2024)	Strong callus induction; somatic embryogenesis depending on transfer medium
Cytokinin + Auxin	BAP + NAA	Wehner & Locy (1981); Seo et al. (2000); Mohammadi & Siviritepe (2007); Migel (2021); Asadi (2023); Sultana et al. (2021)	Direct shoot regeneration, organogenic callus, genotype-dependent responses
	BAP + IAA	Bhardway et al. (2017); Ugandhar et al. (2011)	High bud proliferation and shoot induction
	BAP + 2,4-D	Rajasekaran et al. (1983); Usman et al. (2011)	Callus initiation and embryo formation
	KIN + NAA	Handley & Chambliss (1979)	Regeneration from axillary buds
	BAP + NAA + KIN	Sultana et al. (2021)	Bud regeneration (cotyledons only)
Cytokinin + Additives	BAP + CH	Ahmad & Anis (2005)	Enhanced bud induction and multiplication
PGR media	MS without PGR; MS + KIN	Kiełkowska & Havey (2012)	In vitro flowering; PGR-free media supported normal pollen development

In analyzed studies, cytokinins such as BAP, KIN, ZEA and 2-iP were widely used to stimulate adventitious bud formation and direct shoot regeneration, where BAP has been the most effective for regeneration of multiple explant types. Auxins, particularly 2,4-D, were mostly associated with callusogenesis and somatic embryogenesis, while IAA and NAA are effective as rooting plant growth regulators. The most successful regeneration protocols usually are based on cytokinin–auxin combinations, especially BAP + NAA or BAP + IAA, which supported high-frequency shoot induction in cotyledon, hypocotyl, and nodal explants. More complex PGR combinations, such as BAP + NAA + KIN or BAP + CH, additionally improved bud proliferation in specific genotypes. On the other hand, plant growth regulators-free media or kinetin-only media supported in vitro flowering which indicated that extreme cytokinin–auxin balance may suppress reproductive development. Overall, the diversity of hormonal responses underlines the strong genotype- and explant-specific requirements for efficient cucumber micropropagation.

Micropropagation in cucurbits (*Cucurbita* ssp.)

Table 3 presents an up-to-date chronological order of 11 relevant studies by explant type, medium, plant grow regulator/s combinations and results of cucurbits micropropagation (*Cucurbita* spp.).

Sarowar et al. (2003) developed an efficient in vitro micropropagation protocol for direct shoot growth of pumpkin, using apical buds, which were cultured on MS medium containing BA and NAA as plant growth regulators. The best conditions for shoot regeneration were provided by MS medium supplied with 3.0 mg/l BA. Shoots were efficiently rooted on MS medium supplemented with 1.0 mg/l IBA.

Han et al. (2004) using cotyledon explants and an efficient system for plant regeneration through organogenesis was established. Maximum shoot regeneration was achieved when the proximal parts of the cotyledons of 4-day-old pumpkin shoots were cultured on MS medium with 3 mg/l BA and 0.5 mg/l $AgNO_3$.

Pal et al. (2007) obtained the highest callus induction in MS medium supplemented with 2.5 mg/l 2,4-D in two varieties where the hypocotyl gave a better response compared to the epicotyl.

Haque et al. (2010) aimed to develop appropriate protocols for indirect tissue regeneration via apical meristems. Meristems were isolated on days 15 to 21 from shoots grown in vivo and plated on semi-solid MS medium supplemented with different concentrations and combinations of cytokinin and auxin. Among the different concentrations, the medium with 1.0 mg/l BAP was shown to be the best for callus induction. Calli were subcultured for bud formation in MS medium supplemented with 1.5 mg/l BAP + 0.1 mg/l GA_3. In vitro regenerated buds were rooted on MS + 0.5 mg/l IBA, and were subsequently successfully acclimatized.

Kabir et al. (2010) isolated meristems of different sizes to produce virus-free plants. Meristems with very small sizes produced green, compact callus, but the rate of callus formation was very slow. On the other hand, meristems with large sizes showed a high rate of callus formation, but the calluses were light green and relatively flaky and did not show effective regeneration.

Kim et al. (2010) placed cotyledon explants, cultured on MS medium containing different concentrations and combinations of BA, zeatin, IAA and AgNO3. Efficient bud regeneration was obtained on MS medium, containing 1.0 mg/l zeatin and 0.1 mg/l indole-3-acetic acid.

Sujatha et al. (2013) isolated leaves and nodules that were cultured on MS medium supplemented with 0.5 to 2.5 mg/l TDZ , BAP or KIN for bud induction, and MS supplemented with 1.0 mg/l IBA was used for rooting.

Tuncer (2013) used seeds of ornamental pumpkin (Cucurbita pepo var. ovifera) that were placed on MS medium for germination. From the germinated seeds, they were isolated cotyledons (whole cotyledon and, proximal and distal part of cotyledons) and hypocotyls as initial explants. After four weeks of incubation of the initial explants, they were well-developed calluses obtained. The primordium and calluses were transferred to bud elongation medium with a concentration and combination of 0.1mg /l GA_3 + 1.0 mg /l BAP. Aworunse et al. (2019) in their study described the procedure for in vitro regeneration and the influence of BAP and 2,4-D on pumpkin cotyledons to obtain more shoots.

Table 3. Chronological review of cucurbits micropropagation (*Cucurbita* spp.).

Authors	Type of explant	Medium and PGR combination	Result
Sarowar et al. (2003)	Apical buds	MS medium that contained two plant growth regulators BA and NAA.	The best condition for growth was with 3.0 mg/l BA. They were efficiently rooted on MS medium supplemented with 1.0 mg/l IBA.
Han et al. (2004)	Cotyledons	MS medium with 3 mg/l BA and 0.5 mg/l $AgNO_3$.	Separate shoots were successfully rooted on half strength MS medium with 0.1 mg/l IAA in 2-3 weeks.
Pal et al. (2007)	Hypocotyl and cotyledons	MS medium supplemented with 2.5 mg/l 2,4-D.	The highest callus induction in MS medium supplemented with 2.5 mg/l 2,4-D in two varieties and the hypocotyl was more responsive than the epicotyl.
Haque et al. (2010)	Apical meristem	MS medium, enriched with different concentrations and combinations of cytokinin and auxin.	Among the different concentrations, for callus induction, the medium with 1.0 mg/l BAP proved to be the best.
Kabir et al. (2010)	They isolated meristems of different sizes (0.3-0.5) to produce virus-free plants.	MS supplemented with different concentrations of BAP alone and in combination with NAA, TDZ and 2,4-D.	Very small meristem sizes produced green, compact callus, but the rate of callus formation was very slow, on the other hand, large meristems showed a high rate of callus formation, but the calluses were light green and relatively flaky and did not show effective regeneration.
Kim et al. (2010)	Different parts of cotyledons	MS medium combined with zeatin and IAA.	Efficient bud regeneration was obtained on MS medium. containing 1.0 mg/l zeatin and 0.1 mg/l indole-3-acetic acid.
Sujatha et al. (2013)	Leaf and nodal explants	MS medium enriched with different concentrations of TDZ, BAP or Kn.	TDZ was inferior to BAP or Kn in inducing bud elongation. TDZ at 0.5 mg/L induced maximum number of buds and caused minimal bud elongation in leaf and nodal explants, respectively, after 4 weeks of culture.
Tuncer (2013)	Proximal part, distal part, entire cotyledons and hypocotyl	MS medium and 36 different hormone combinations for callus proliferation and shoot regeneration.	highest average number of shoots per explant was obtained from 0.5 mg/l BAP + 1.0 mg/l kinetin in hypocotyl and proximal explants.
Aworunse et al. (2019)	Induction of multiple shoots from cotyledons of pumpkin	MS medium with different concentrations of BAP.	The most effective medium was found to be a concentration of 0.45. mg/l BAP.
Sarkar et al. (2021)	Apical buds and nodal segments	Different concentrations of auxins , cytokines and gibberellins were used alone or in combination supplemented in MS medium.	shoots was also obtained in node explants on MS medium containing 2.0 mg/l BAP + 0.1 mg/l NAA.
Delgado-Paredes et al. (2023)	Node and leaf segments	MS with 0.02 mg/l IAA, 0.5 mg/l BAP and 0.02 mg/l GA_3.	Micropropagation was achieved in all of these species.

The explants were cultured on MS medium with different concentrations of BAP. The medium with a concentration of 0.45 mg/l BAP was found to be the most effective.

Sarkar et al. (2021) used basal medium to stimulate micropropagation from apical buds and nodal segments. The explants were cultured on different MS media using growth regulators of BAP, NAA, in each medium. For rooting, shoots were isolated and cultured in complete MS medium supplemented with 0.5, 1.0 and 2.0 mg/l or IBA, NAA, IAA.

Delgado-Paredes et al. (2023) cultivated apical buds and nodal segments in ex vitro conditions. The explants were placed on several MS media containing IAA or NAA, BAP or MS supplemented with 0.25% activated carbon. Micropropagation was achieved in the species *C. maxima* and *C. moschata* that were the subject of the research.

Table 4. Summary of plant growth regulators used in cucrbits (*Cucurbita* spp.) micropropagation and their regenerative outcomes across different studies.

PGR type	PGR used	Study	Main results
Cytokinins only	BA	Sarowar et al. (2003); Han et al. (2004); Sujatha et al. (2013); Aworunse et al. (2019); Sarkar et al. (2021)	Efficient direct shoot regeneration from apical buds; high organogenesis from cotyledons; bud induction from leaves and nodes; optimal shoot proliferation at 0.45–3 mg/l BA
	KIN	Sujatha et al. (2013)	Moderate bud induction from leaves and nodal explants
	ZEA	Kim et al. (2010)	Strong bud regeneration from cotyledon explants at 1 mg/l zeatin
	TDZ	Sujatha et al. (2013)	High cytokinin-driven bud induction and shoot formation
Auxins only	2,4-D	Pal et al. (2007)	Strong callus induction from hypocotyl and epicotyl explants
	IAA	Delgado-Paredes et al. (2023)	Micropropagation of C. maxima and C. moschata from apical buds/nodal segments
	NAA	Delgado-Paredes et al. (2023); Sarkar et al. (2021)	Root induction and shoot establishment
	IBA	Sarkar et al. (2021)	Efficient rooting of regenerated shoots
Cytokinin + Auxin	BA + NAA	Sarowar et al. (2003); Sarkar et al. (2021)	Direct shoot regeneration from apical buds; stimulation of shoot multiplication
	BA + IAA	Kim et al. (2010); Delgado-Paredes et al. (2023)	Zeatin/BA + IAA combinations enhanced bud regeneration in cotyledons and nodal segments
	BA + GA_3	Haque et al. (2010); Tuncer (2013)	Bud formation and elongation from meristem- or callus-derived tissues
	Zeatin + IAA	Kim et al. (2010)	Optimal bud regeneration from cotyledon explants
	BAP/TDZ/KIN + various auxin	Sujatha et al. (2013)	Broad organogenic responses depending on cytokinin type and dose
Cytokinin + Additives	BA + $AgNO_3$	Han et al. (2004); Kim et al. (2010)	Increased shoot regeneration; suppression of ethylene-related inhibition
	BA + Activated carbon	Delgado-Paredes et al. (2023)	Enhanced shoot growth and reduced phenolic oxidation

Cucurbits micropropagation commonly utilizes shoot tips, nodal segments, cotyledonary nodes, and hypocotyl explants due to their high regenerative potential and responsiveness to hormonal stimulation. A comparison of published micropropagation studies for Cucurbita spp. highlights that plant growth regulators play a critical role in determining the morphogenetic response (Table 4).

Cytokinins such as benzyladenine (BA), kinetin (KIN), and zeatin are the primary growth regulators used to induce shoot proliferation, while auxins including indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), and indole-3-butyric acid (IBA) support rooting and, when combined with cytokinins, increase organogenic responses. Optimal regeneration is typically achieved with BA-based cytokinin treatments alone or in low-auxin combinations, which effectively promote shoot induction across most cucurbit species (Table 4).

CONCLUDING REMARKS

Micropropagation of cucumber (Cucumis sativus L.) and cucurbits (Cucurbita spp.) has advanced significantly, providing efficient strategies for large-scale propagation and genetic improvement. Success depends on explant type, genotype, and plant growth regulators balance, with cytokinins, particularly BAP, being most effective for shoot induction, and auxins such as 2,4-D and NAA essential for callusogenesis and rooting. Cotyledons and hypocotyls show high morphogenetic potential in cucumber, while shoot tips and nodal segments are most responsive in cucurbits. In cucurbits, optimal regeneration is achieved through cytokinin-auxin combinations, with BA-based treatments and IBA supporting rooting. These findings highlight the importance of genotype-specific, reproducible protocols to enhance scalability and ensure genetic reliability. Micropropagation remains a critical tool for producing disease-free plants and accelerating classical breeding programs.

Future research in micropropagation of cucumber and cucurbits shall be directed in investigation of molecular markers and genomic tools to predict genotype-specific responses to plant growth regulators as well as study of novel combinations of plant growth regulator and signaling pathways to improve morphogenesis and reduce variability. The new challenges in agricultural production shall integrate micropropagation with abiotic and biotic stress resistance breeding for climate-resilient cultivars, while the commercial laboratories shall develop automated culture systems and bioreactors to lower production costs and improve scalability in micropropagation. Finally, the new era in biotechnology will combine micropropagation with genetic transformation, CRISPR-based genome editing, and somatic embryogenesis for advanced crop improvement.

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ХРОНОЛОШКИ ПРЕГЛЕД НА МИКРОПРОПАГАЦИЈАТА НА КРАСТАВИЦА (*Cucumis sativus L.*) И ТИКВИ (*Cucurbita spp.*)

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Резиме

Микропропагацијата е клучен биотехнолошки метод за брзо и обемно размножување на градинарски култури. Денес, микропропагацијата игра клучна улога во производството на растенија без болести, зачувувањето на генетските ресурси и претставува основна алатка за програмите за селекција на култури. Краставицата (*Cucumis sativus L.*) и тиквите (*Cucurbita spp.*) се економски важни видови кои се опширно проучувани за in vitro регенерација. Овој прегледен труд ги синтетизира протоколите објавени во текот на изминатите децении, фокусирајќи се на типот на експлант, медиумот за култура и комбинациите на регулатори за раст на растенијата. Анализирани се вкупно 29 трудови за краставица и 11 трудови за тикви за да се идентификуваат истражувачките трендови со текот на времето и критичните фактори што влијаат на морфогенезата. Цитокинините, особено BAP, биле постојано ефикасни за индукција на адвентивни изданоци кај краставицата, додека ауксините како што е 2,4-D ја промовираат калусогенезата кај краставица. Оптималната регенерација на краставица честопати се потпира на комбинации на цитокинин-ауксин, при што котиледоните и хипокотилите покажуваат висок морфогенетски потенцијал. Кај тиквите, врвовите на изданоците, нодалните сегменти и котиледоните биле најреспонсивни експлантати, каде третманите базирани на BA постигнале супериорна пролиферација на изданоците, а IBA поддржува ефикасно вкоренување. Генотип-специфичните одговори и хормоналната рамнотежа се критични фактори за успешна микропропагација кај двете култури. Добро дизајнираните протоколи за микропропагација се од суштинско значење за ефикасна регенерација и генетско подобрување на двете култури. Разбирањето на одговорите специфични за експлантот и зависноста од генотипот во однос на регулаторите за раст на растенијата може да ја подобри репродуктивноста и скалабилноста на in vitro системите, отворајќи го патот за напредна селекција и биотехнолошки апликации.

Клучни зборови: *култура на растителни ткива, растителни регулатори на раст, експланти, органогенеза, калусогенеза, регенеранти.*