



## **Auxins and Cytokinins Involved in Micropropagation of Pepino Plant (*Solanum muricatum* Aiton)**

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### **Abstract**

A reliable and successful micropropagation protocol was developed for pepino plant (*Solanum muricatum* Aiton) from nodal segment explants grown on MS medium. The best values of shoot multiplication traits were recorded from the addition of 3 mg.l<sup>-1</sup> kinetin by producing 2.3 shoots/explant, 3.6 cm and 9.6 leaves/ explant which was significantly superior upon the addition of BA at the same levels. In case of adding 3 mg.l<sup>-1</sup> kinetin, the best root formation attributes were achieved from the use of 0.2 mg.l<sup>-1</sup> IAA that resulted a maximum number of roots (14.33 roots/ explant). The longest root length (15.33 cm) was achieved when 0.3 mg.l<sup>-1</sup> IAA was used. A 100% rooting percentage was recorded from the all tested auxins including IAA, IBA and NAA. The gradually moved plantlets from the heterotrophic phase in the lab to the autotrophic phase in the greenhouse showed 100% success. The plantlets did not show any abnormal growth or morphological changes. It is concluded that this important plant can be easily propagated by tissue culture technique through a reliable micropropagation protocol.

**Keywords:** IAA, IBA, Kinetin, Micropropagation, NAA, Pepino, *Solanum muricatum* Aiton

### **Introduction**

Pepino dulce is the common name of *Solanum muricatum*, it belongs to the family *solanaceae* and genus *Solanum* (Ipgri and Comav, 2004). It is one of the subtropical evergreen shrubs natively present in several countries of South America. It is commercially propagated in Australia, USA, New Zealand, Turkey, etc. (Aghdaei *et al.*, 2019). It comes in different colors and shapes. The name pepino comes from the Spanish language which means "cucumber" because it is similar to cucumber in texture and taste especially at the premature stage but when it matures, the taste becomes sweet that is why it is called sweet cucumber (Orhan *et al.*, 2014).

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This plant is widely used as an ingredient in many food meals, salads and desserts (Prohens *et al.*, 1996). Pepino fruit contains water (92%). It is rich in potassium and vitamin C about 1000 mg.kg<sup>-1</sup> and 200 mg.kg<sup>-1</sup> respectively and with a reasonable amount of thiamine and minerals (Pluda *et al.*, 1993, Sánchez *et al.*, 2000, Sudha *et al.*, 2011 and Aghdai *et al.*, 2019). In spite of its nutritive value, it has been proved that this plant has several medicinal values such as it relieve hypertension act as antioxidant, antitumoral and diuretic (Cavusoglu, and Sulusoglu 2013) as well as it is widely used as Hepatoprotective. Pepino is a crop that is usually grown in the greenhouse and when compared with other greenhouse crops, it has a longer growing season with a yield about 75 tons.ha<sup>-1</sup> (Prohens and Nuez, 1999). Pepino could be propagated either by seeds or vegetatively by cuttings. However, natural propagation via seed has many problems as a low rate of seed germination with low quality plants and high heterozygosity levels (Nemati and Tehranifar, 2007). To overcome these problems it could be propagated via tissue culture from vegetative parts which produce seedlings with high quality and homozygosity levels (Jordan *et al.*, 1990a, 1990b and Jordan *et al.*, 1993). Moreover, micropropagation is a great tool used in plant breeding fields as gene transformation and intergeneric hybrids (Sakomoto and Taguchi, 1991). Aysun and Melekber (2013) developed a protocol for Pepino micropropagation from leaves and nodes. They tested different combinations of BA, NAA and IBA on pepino plantlets grown on MS medium.

The objective of the present study was the micropropagation of pepino plant from nodal segments for mass production of healthy and vigorous transplants as well as to introduce this new plant to our farming community and the general public.

## Materials and Methods

Nodal segments of pepino were taken from mature plants as explants and they were surface sterilized by 10% of commercial bleach (NaOCl) for 10 minutes then thoroughly washed 3 times with sterilized dist. water each for three minutes. Then the nodal segments were cultured on MS Basal Medium (Murashige and Skoog, 1962). For multiplication stage MS medium was supplemented with BA (0, 1, 2 and 3) mg.l<sup>-1</sup> and kinetin (0, 1, 2 and 3) mg.l<sup>-1</sup>. Furthermore, at rooting stage three kinds of auxins were tested including NAA, IBA and IAA at 0, 0.1, 0.2 and 0.3 mg.l<sup>-1</sup> with 0.5 mg.l<sup>-1</sup> BA or 3 mg.l<sup>-1</sup> Kinetin. Data were recorded on the shoots number/ explant, leaves number/ explant and mean length of shoots (cm) for the shoots multiplication stage. For the rooting stage, roots number/ explant, mean length of roots (cm) and the percentage of rooting (%) was determined in response to various treatments. The explants disinfection and culture process were done under the aseptic conditions of the air-flow cabinet. The cultures were kept at the growing room area under controlled conditions at 25± 2°C and light illumination of 16 hrs light and 8 hrs darkness under 1000 Lux light intensity.

## Results and Discussion

Almost 100% of healthy cultures were achieved from the initiation stage of pepino nodal segment explants grown on MS medium after a successful disinfection with NaOCl (10%) for 10 minutes (Fig.1, A). These results assure the vital influence of sodium hypochlorite being the best option for getting healthy in vitro culture (Toma, 2019). Table (1) illustrates the influence of BA and kinetin on the shoot multiplication stage of pepino explants cultured on MS medium after six weeks in culture. It is clear from the table that the best results were achieved when 3

mg.l<sup>-1</sup> kinetin was used which resulted in the maximum number of shoots explant-1 (2.3) shoots/explant, shoot length (3.6 cm) with the highest number of leaves explant-1 (9.6). While the least number of shoot per explant and the shortest mean length were acquired when 3 mg.l<sup>-1</sup> BA was added by producing 1 shoot and 0.46 cm respectively. While the lowest number of leaves per explant was obtained when 2 mg.l<sup>-1</sup> IBA was used with 4.3 leaves/ explant. These results are in agreement with what has been reported by Aysun and Melekber (2013) that cytokinins are essential for inducing pepino shoot multiplication. The roles of cytokinins is well known in enhancing shoot multiplication by their positive actions on breaking apical dominance, bud flushing up and proteins synthesis. Thus, adding cytokinins usually promote shoot multiplication of explants grown on culture media supplemented with propal concentrations (Aysun and Melekber, 2013).

**Table 1. Influence of BA and kinetin on shoot multiplication stage of Pepino explants grown on MS medium after six weeks in culture**

Treatments	Number of Shoots/ Explant	Shoots length (cm)	Number of Leaves/ Explant
0.0	1.3 b	2.83 b	7.6 b
BA (1.0 mg.l <sup>-1</sup> )	1.3 b	1.00 c	4.67 d
BA (2.0 mg.l <sup>-1</sup> )	1.3 b	0.92 c	4.3 d
BA (3.0 mg.l <sup>-1</sup> )	1.0 c	0.46 cd	2.6 e
Kinetin 1.0 mg.l <sup>-1</sup> )	1.6 b	2.16 b	6.3 c
Kinetin 2.0 mg.l <sup>-1</sup> )	1.6 b	2.83 b	6.6 c
Kinetin 3.0 mg.l <sup>-1</sup> )	2.3 a	3.6 a	9.6 a

Table 2 shows the influence of IAA, IBA and NAA on the rooting stage of pepino shoots grown on MS medium enriched with 3.0 mg.l<sup>-1</sup> kinetin after six weeks in. In general, IAA was better than the rest of auxins by producing better rooting parameters. The highest number of roots per explant was obtained when 0.2 mg.l<sup>-1</sup> IAA was added by producing 14.33 roots/ explant. However, the longest mean of the root was obtained when 0.3 mg.l<sup>-1</sup> IAA was added by giving 15.33 cm as mean shoots length. Moreover, the highest rooting rate (100%) was acquired when IAA at (0.1, 0.2 and 0.3) mg.l<sup>-1</sup>, IBA at 0.3 mg.l<sup>-1</sup> and NAA at 0.2 and 0.3 mg.l<sup>-1</sup> were used while the other gave 90%. On the other hand the control treatment gave the lowest rooting parameters which were 3.33 roots/ explant, 4.55 cm mean shoot length and 30% rooting percentage.

**Table 2. Influence of IAA, IBA and NAA on the rooting stage of Pepino shoots grown on MS medium enriched with 3.0 mg.l<sup>-1</sup> Kinetin after six weeks in culture**

Treatments	Roots Number/ Explant	Roots length (cm)	Rooting Percentage (%)
0.0	3.33 e	4.55 g	30 c
IAA (0.1 mg.l <sup>-1</sup> )	8.33 d	13.67 b	100 a
IAA (0.2 mg.l <sup>-1</sup> )	14.33 a	15.00 a	100 a
IAA (0.3 mg.l <sup>-1</sup> )	12.67 b	15.33 a	100 a
IBA (0.1 mg.l <sup>-1</sup> )	10.33 c	9.33 c	90 b
IBA (0.2 mg.l <sup>-1</sup> )	8.67 d	7.00 f	90 b
IBA (0.3 mg.l <sup>-1</sup> )	11.00 b	9.33 c	100 a

NAA (0.1 mg.l <sup>-1</sup> )	11.67 b	7.67 de	90 b
NAA (0.2 mg.l <sup>-1</sup> )	10.00 c	8.83 d	100 a
NAA (0.3 mg.l <sup>-1</sup> )	13.33 a	7.83 e	100 a

Table 3 illustrates the effect of IAA, IBA and NAA on the rooting stage of Pepino shoots grown on MS medium enriched with 0.5 mg.l<sup>-1</sup> BA after six weeks in culture. Overall, the culture performance was better in the presence of 0.5 mg.l<sup>-1</sup> BA, IBA and NAA than IAA by giving the best results for rooting parameters. Accordingly, the highest number of roots/ explant was obtained when 0.1 mg.l<sup>-1</sup> NAA was added (11.33 roots/ explant). Furthermore, the longest roots were achieved when 0.3 mg.l<sup>-1</sup> IBA was added by giving 19.67 cm. Moreover, the highest rooting percentage was acquired when 0.1, 0.2 and 0.3 mg.l<sup>-1</sup> IBA were used by reaching 100%. Nevertheless, the lowest number of roots and rooting percentage were obtained for control treatment which gave only 3.33 roots/ explant and 30% rooting percentage. While the shortest roots were acquired when 0.3 mg.l<sup>-1</sup> NAA was added by producing a 3.67 cm root length. These results confirm the importance of auxins in inducing adventitious root formation on micro-shoots (Toma, 2019).

**Table 3. Influence of IAA, IBA and NAA on the rooting stage of Pepino shoots grown on MS medium enriched with 0.5 mg.l<sup>-1</sup> BA after six weeks in culture**

Treatments	Roots number/ Explant	Roots length (cm)	Rooting Percentage (%)
0.0	3.33 e	4.55 f	30 e
IAA (0.1 mg.l <sup>-1</sup> )	6.33 c	17.83 b	80 d
IAA (0.2 mg.l <sup>-1</sup> )	8.33 b	14.50 c	85 c
IAA (0.3 mg.l <sup>-1</sup> )	10.67 a	13.00 d	90 b
IBA (0.1 mg.l <sup>-1</sup> )	7.33 b	16.33 b	100 a
IBA (0.2 mg.l <sup>-1</sup> )	7.67 b	16.50 b	100 a
IBA (0.3 mg.l <sup>-1</sup> )	7.67 b	19.67 a	100 a
NAA (0.1 mg.l <sup>-1</sup> )	11.33 a	14.33 c	80 d
NAA (0.2 mg.l <sup>-1</sup> )	8.33 b	8.83 e	80 d
NAA (0.3 mg.l <sup>-1</sup> )	7.67 b	3.67 g	85 c

The produced plantlets were carefully acclimatized and gradually moved from the lab conditions to the greenhouse with a high survival rate (100%). The acclimatized plantlets were grown normally and showed no abnormal characteristics in their morphology (Fig. 1).



**Figure 1.** Micropropagation stages of Pepino explants grown on MS medium: A: Explants initially inoculated on the medium. B: Six weeks growing explants on shoot multiplication medium. C: Well rooted shoots at root formation stage. D: The best rooting treatment (Kinetin 3.0 mg.l<sup>-1</sup>) vs. Control. E: The beginning of hardening stage. F: Well acclimatized Pepino plantlets under greenhouse conditions.

## Conclusions

In conclusion. This newly introduced plant to the Kurdistan Region of Iraq can be readily propagated by tissue culture technique and it is highly recommended to be commercially produced towards mass production to meet the local community demand.

## Conflict of interest

The authors declare that they have no competing interests.

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