Effect of different concentrations of auxin as well as cytokinin on shoot initiation, formation and multiplicationof pepino (*Solanum muricatum*Ait.) cv. Valentiawith MS semi-solid medium

ABSTRACT

The pepino (*Solanum muricatum* Aiton) is conventionally propagated through vegetative means. But, this approach of propagation is not feasible for commercial production of plants and has at more time the chances of reduction yield. Therefore, the availability of quality planting material is of urgent need. Recently, there has been a promising approach for pepino multiplication at large scales through tissue culture because it generates a large number of contamination free plantlets in a minimum space and time. The combination of different growth regulators may be tried to maximize the initiation, growth and development of shoot in commercial propagation of pepino plants. The Minimum days taken for shoot initiation (5.21 days) was noted under BAP 3.00 mgl-1 + IBA 0.50ppm, and minimum days taken for shoot development (40.82days) was noted under of BAP 3.00ppm + IBA 1.00ppm. The highest length of shoot (9.52cm) was produced in media supplemented with BAP 3.00 ppm + IBA 1.00ppm. Maximum percent of developed shoots obtained from the established culture (67.79) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm; While maximum shoots per explants/ innoculums from the established culture (4.06) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm. Maximum number of leaves / plantlet from the established culture (10.85) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm and maximum fresh weight (mg) of shoot / plantlet obtained from the established culture (26.05mg) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm.

KEYWORD: Commercial propagation, *Solanum muricatum, herbaceous crop, antioxidant effects*

1. INTRODUCTION

Introduced and established crops have a vital role in human progress in any region of the world, many of the most important crops do not come from these regions, but were new crops at the time of their introduction. However, many underutilized or neglected crops and non-domesticated species need to be explored and could be developed as new crops in many regions around the world. One of the crops of the Solanaceae family is Pepino (*Solanum muricatum* Aiton), a neglected crop that was very important in the Andean region in pre-Columbian times. Pepino is a herbaceous crop that produces fleshy berries weighing 100 to 700 g, round, ovoid or

elongated, with a normally golden yellow skin. The flesh is yellow, juicy, and has a sub-acid, mild flavor (Levy*et al.*, 2006). It is visually attractive; the skin is usually golden yellow and is covered with purple stripes.Pepino is completely edible, including the skin and seeds, so it tastes like a mix between a pear and a banana. Although it is sexually fertile and highly heterozygous by seed propagation, it can also be propagated vegetative (normally by cuttings). Although is a native to South America, this crop has become domesticated in other parts of the world such as the Netherlands, New Zealand and Spain (Blanca *et al.*, 2007). It is also valued for its medicinal applications. The aqueous extract of its fruits could moderate the progression of diabetes due to its anti-inflammatory, anti-glycative and antioxidant effects (Hsu*et al.*, 2011). A medium serving (~100 g) of its fruit provides 80 calories of energy and 5 g of oatmeal-like fiber that helps lower cholesterol and is easy to digest. Fiber also helps with constipation and tends to soothe stomach ulcers as well. The fruit is rich in minerals and vitamin C, but with a low content of starch, sugars and no oxalates. The minerals contained in Pepino fruits are Fe, Zn, Cu, Mn, Ca & P. It has been observed that the level of glucose and fructose decreases during ripening, while the concentration of sucrose increases as ripening progresses. A distinct decrease in protein and fat content has also been noted as the fruit changes from unripe to ripe (Huyskens*et al.*, 1999). Pepino is known as a source of beta-carotene, 27 mg per 100 grams of fruit pulp. The crop is also considered a reservoir of sucrose during the final ripening stage. Fruits picked unripe are tasteless and non-aromatic.Uses of the pepino plant include juices, preserves, ice-cream, and jam. Fruits at the “green” and “turning stage” before ripening may have a cucumber-like scent and can be used in green salads or as a vegetable in stews, and it can be consumed as a refreshing dessert fruit or as an ingredient of fruit salads (Pandey*et al.*, 2021).Effect of different concentrations of auxin as well as cytokinin in tissue culture enables the production of large number of plants through mother plant. It enables the pepino plant to produce large number of plants with desired traits quickly. It ensures that there is no genetic variation because, it is carried out under *in vitro* and sterile conditions, which means that it kills any pathogen, pest or disease already contained on the plant therefore, reducing the risk of the parent plant transferring diseases to the offspring. Viewing the above motto the present study effect of different concentrations of auxin as well as cytokinin on shoot initiation, formation and multiplication with MS semi-solid medium.

2. MATERIALS AND METHODS

The explants were cut into suitable segment using surgical blade and thoroughly washed under running water. After that explants carefully rinsed with a mild extran solution for 2 minutes and then carefully washed with distilled water 4 to 5 times then explants were cut by reducing the size and removing excessive plant material after that explants were soaked in different sterilization treatment with various time duration. After all sterilization procedures, explants are introduced into the MS medium with different concentration of auxins and cytokinin. The culture test tube were kept under continuous cool and white fluorescent tube light (1500 Lux) at a temperature of 25 ± 2^0C. The new glass-wares were cleaned by washing them in a solution of 10.0% High Spark Cleaning Solution (Himedia) in hot water. The glass-wares were then soaked in the solution of chromic acid for overnight and rinsed thoroughly with hot water. Finally, rinsed with double distilled water and dried at 150ºC in hot air oven for an hour. The molten warm culture medium was disposed offinto empty culture vessels. The culture vessels were washed with hot water containing 10.0 per cent High Spark Cleaning Solution (Himedia) and rinsed with distilled water followed by sterilization in hot air oven at 150ºC for one hour.

2.1 Preparation of explants for culture: Attempts have been made for the induction of callus using internode cutting (2.0 cm) and leaves (1.5 cm) of pepino plant explants. Explants were washed thoroughly under running tap water for about half an hour to remove the soil and dust particles from the plants. After that explants were treated with 2 percent Extran (Merck) for about one to two minutes with constant shaking and then thoroughly washed with sterilized distilled water to make the plant material free from superficial contamination. Rest of the sterilization procedure was carried out in laminar air flow cabinet. Explants were transferred to laminar air flow and rinsed the explants with 70 percent alcohol for about 1-2 minutes. There after washed the explants with autoclaved distilled water 3 to 4 times to remove the alcohol. After that five treatments were used for explants sterilization for different time duration as described in (table-1) the experiment was conducted with three replications including 10 pots each treatment and carried out in CRD design.

Table 1.Chemical Treatments used for the study

Sr. No.	Chemical Treatment	Percent	Treatment Time
1.	Mercuric Chloride ($HgCl_2$)	0.1%	Control, 1.5, 3.0, 4.5, 6.0 minutes
2.	Ethanol	70%	Control, 1.5, 3.0, 4.5, 6.0 minutes

3.	Mercuric Chloride + Ethanol	0.1+70%	Control, 1.5, 3.0, 4.5, 6.0 minutes
4.	Sodium Hypochlorite (NaOCl)	1.0 %	Control, 1.5, 3.0, 4.5, 6.0minutes
5.	Mercuric Chloride ($HgCl_2$)+(Bavistin)	0.1%+0.15	Control,1.5,3.0,4.5,6.0minutes

2.2 Preparation of MS culture media from the stocks: To prepare one liter of MS media, the 100 ml of stock solution of macro-nutrients (stock A), 20 ml of stock solution of micro-nutrients (stock B), 10 ml of stock solution of Fe-EDTA (stock C), 100 mg/l of myo-inositol and 20 ml of each of the stock solution of vitamins were added to 1litre volumetric conical flask and 500ml of distilled water was added. Thereafter, 30.0 g/l sucrose was added to mentioned flask. Different concentrations of hormonal supplements as required were added either in single or in different combinations to this solution and were mixed thoroughly.The whole mixture was then made up to 1000ml with further addition of distilled water. The pH of the medium was adjusted to 5.8 using a digital pH meter with addition either of 1N NaOH/1N HCl. The solution was then made up to 1 litre with adding distilled water. Finally, 8g agar was added to 1litreof media and the whole mixture was then gently heated on a hot plate. The warm medium was dispensed into culture vessels. The culture vessels were plugged with plastic cap and marked with a glass marker to indicate specific treatment.

2.3 Sterilization of the culture media: The culture media were sterilized in autoclave at a pressure of 15 lbs per $inch^2$ at 121.6 ºC for 25 minutes. After cooling, the media were stored in dark condition at 25±2ºC for about a week.

2.4 Inoculation of explants: The explants were cut vertically and horizontally, into smaller pieces (1.5mm) with the surgical blade and were carefully cultured in the test tubes containing the medium. Single explant was cultured test tube containing 15 ml of MS medium supplemented with BAP and IBA growth regulator different concentrations.

2.5 Culture incubation: The prepared culture test tubes after inoculation were kept in culture room at 25±2°Ctemperature for callusing. The explants were incubated for shoot induction and proliferation and maintained under light intensity of 30.0 µm $dm^{-1}s^{-1}$ illuminated with white light florescence tubes, 25±2°C temperature, humidity at 65 per cent and photoperiod (2000- 3000 lux) of 16 hours light and 8 hours dark in culture room.

2.6 Data analysis: For statistical analysis of data generated through various experiments was qualified and the significance of difference among means were determined by ANOVA using Window stat 9.2 software

2.7Data observations: To assess the effects of different treatments, in order to estimate *in-vitro* multiplication rate of (*Solanum muricatumAit.*) cv. Valentiathe mean value of each parameters were used.

1. **Days taken for shoot initiation for pepino:** The data on days for shoot initiation from greening of explants were also recorded regularly.
2. **Day taken for shoot development:** The data on days for shoot regeneration from meristem segments were also recorded.
3. **Length of shoot (cm):** The length of shoots proliferated were recorded the mean value of the data provided the shoot length.
4. **Percent shoot development:** The percentage of shoot formation from callus was observed after inoculation by using the following formula:

$$\text{Percent shoot development} = \frac{\text{Number of calli showing shoot}}{\text{Number of calli inoculated}} \times 100$$

5. **Number of shoots per explants/ inoculums:** The number of shoots proliferated were recorded and the mean value of the data provided the number of shoot.
6. **Number of leaves/plantlet:** The number of leaves was counted from base to tip from the single stem plantlet.

3. RESULT

3.1 Number of days taken for shoot initiation in pepino

The data explain that optimum days taken for shoot initiation from the established explants in the culture medium was significantly increased from 5.27 to 11.93 days with the addition of BAP 3.0ppm + IBA 1.0ppm and BAP 1.50ppm + IBA 0.50ppm respectively (Table no. 2 and Fig. no. 1). Maximum days taken for shoot initiation from the established culture (11.93days) was noted under the treatment of BAP 1.50ppm + IBA 0.50ppm; followed by (11.53), (11.03) and (10.38) days with the treatments of BAP 4.50ppm + IBA 0.50ppm, BAP 4.50ppm + IBA 1.50ppm and BAP 1.50ppm + IBA 1.50ppm respectively while the minimum days taken for shoot initiation (5.21 days) was noted under BAP 3.00 mgl^{-1} + IBA 0.50ppm.A critical observation was recorded as the maximum days taken for shoot initiation in the culture (11.93 days) was recorded under the treatment of BAP 1.50 ppm + IBA 0.50ppm; while the minimum days taken for shoot development (5.27 days) was observed under BAP 3.00ppm + IBA 1.00ppm.

Table: 2. Number of days taken for shoot initiation for pepino plant

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	11.93	7.35	11.53
IBA 1.00 ppm	9.11	5.27	8.47
IBA 1.50 ppm	10.38	6.69	11.03
Gen. Mean	10.47	6.43	10.34
C.D.	2.08	0.59	2.08
SE(m)	0.59	0.16	0.59
SE(d)	0.83	0.23	0.83
C.V.	9.76	4.50	9.89

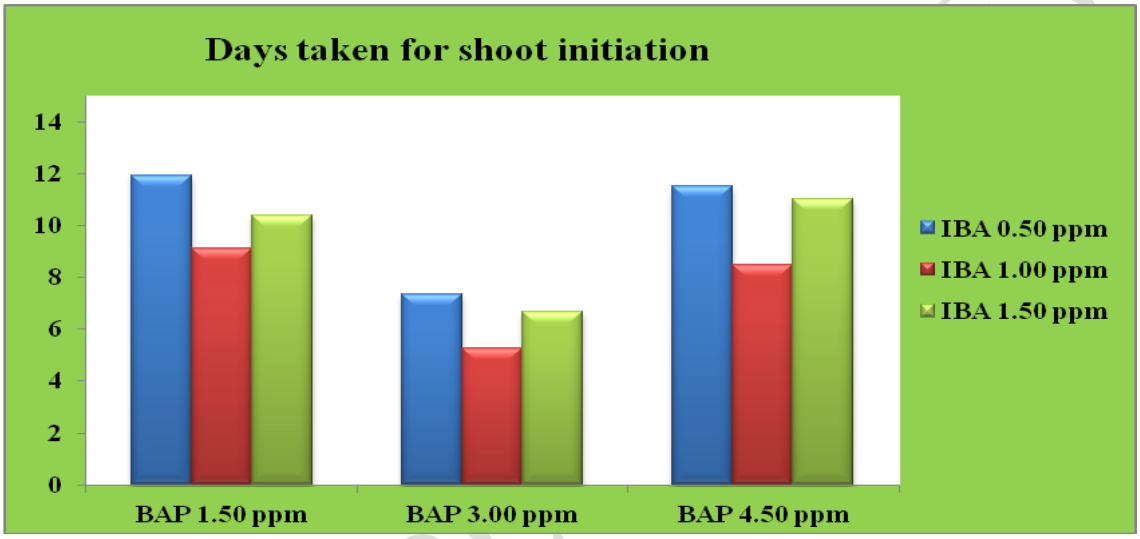

Fig. 1. Number of days taken for shoot initiation for pepino plant

3.2. Days taken for shoot development in pepino plantlets

The effect of different plant growth regulators combinations on days taken for shoot development were studied which had been clearly represented in the (Table no. 3 and Fig. no. 2). Maximum number of day taken for shoot development from the established culture (52.54 days) was noted under the treatment of BAP 4.50ppm + IBA 0.50ppm followed by (51.66), (51.47) and (49.28) days with the treatments of BAP 1.50ppm + IBA 0.50ppm, BAP 4.50ppm + IBA 1.50ppm and BAP 1.50ppm + IBA 1.50ppm respectively while the minimum days taken for shoot development (40.82 days) was noted under of BAP 3.00ppm + IBA 1.00ppm. A critical observation was recorded as the maximum days taken for shoot development in the culture (52.54 days) was recorded under the treatment of BAP 4.50 ppm + IBA 0.50ppm; while the minimum days taken for shoot development (40.82 days) was observed under BAP 3.00ppm + IBA 1.00ppm.

Table: 3. Days taken for shoot development for pepino plant

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	51.66	49.19	52.54
IBA 1.00 ppm	45.53	40.82	48.57
IBA 1.50 ppm	49.28	46.12	51.47
Gen. Mean	48.82	45.38	50.86
C.D.	1.92	3.95	1.99
SE(m)	0.54	1.12	0.56
SE(d)	0.77	1.58	0.80
C.V.	1.93	4.27	1.92

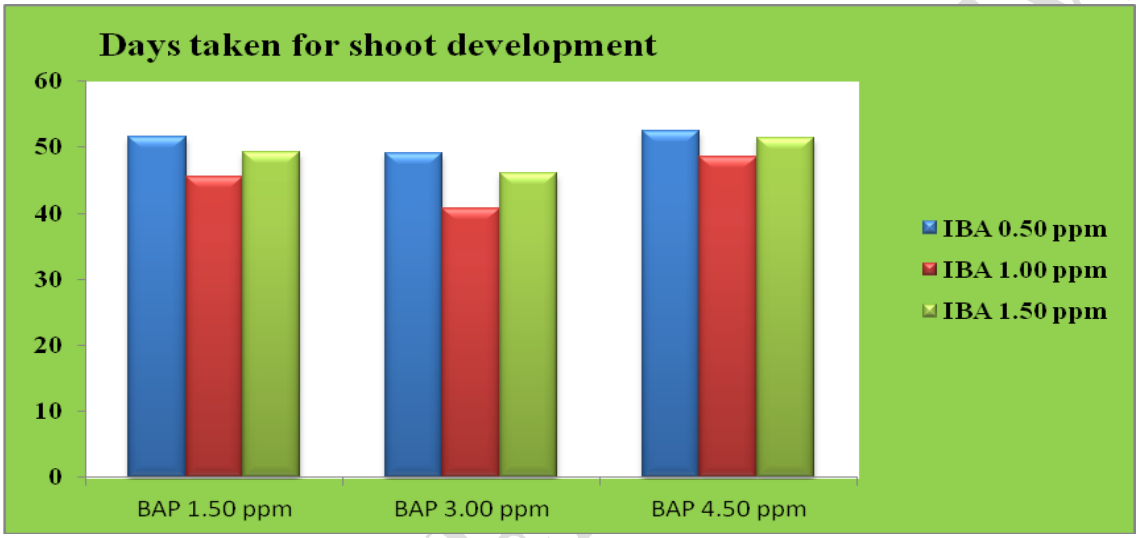

Fig.2.Days taken for shoot development

3.3. Length of shoot (cm) of pepino plantlets It is evident from data in the (Table no. 4 and Fig. no. 3) that there is a significant difference amongst different BAP and IBA combination concentrations. The highest length of shoot (9.52cm) was produced in media supplemented with BAP 3.00 ppm + IBA 1.00ppm and lowest length of shoot (2.47cm) belonged to media with BAP 1.50ppm + IBA 0.50ppm; followed by (3.69cm), (4.62cm), (4.66cm) with BAP 1.50ppm + IBA 1.50, BAP 4.50ppm + IBA 0.50, BAP 1.50ppm + IBA 0.1.00 respectively.

Table: 4. Length of shoot in (cm) for pepino plantlets

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	2.47	7.71	4.62
IBA 1.00 ppm	4.66	9.52	5.27
IBA 1.50 ppm	3.69	7.16	7.60
Gen. Mean	3.61	8.13	5.83
C.D.	1.58	1.50	1.50
SE(m)	0.44	0.42	0.42
SE(d)	0.63	0.60	0.60
C.V.	21.46	9.09	12.67

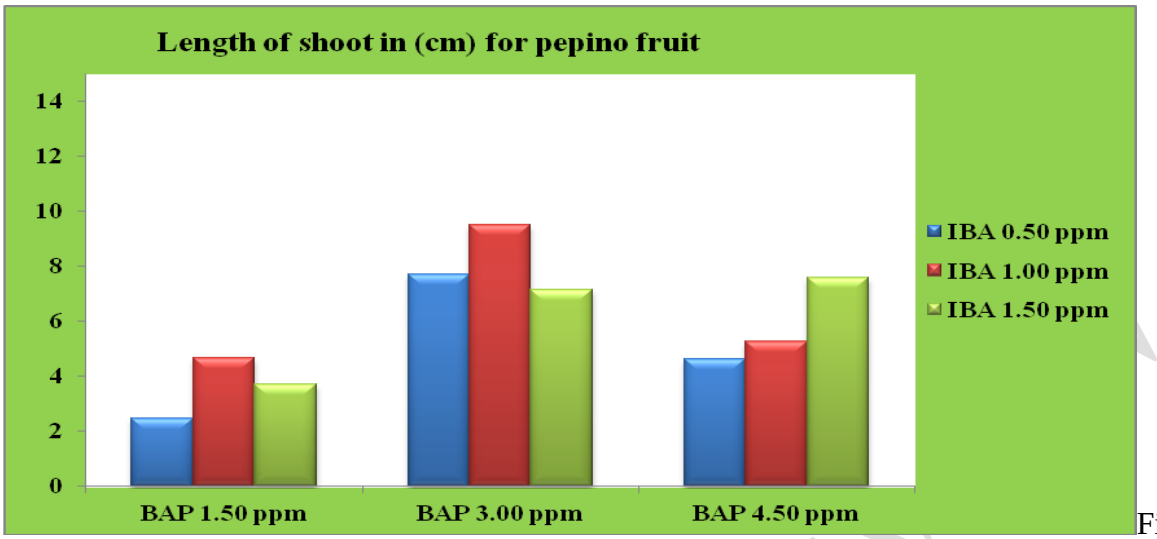

Fig. 3.Length of shoot in (cm) for pepino plantlets

3.4 Percentage of shoot development of pepino plantlets

The (Table no. 5 Fig. no. 4) explains that percent of shoot development obtained from the established explants in the culture medium was significantly increased from (30.06) to (67.79) with the addition of BAP 1.50ppm + IBA 0.50ppm and BAP 3.00ppm + IBA 1.00ppm media, respectively. Maximum percent of developed shoots obtained from the established culture (67.79) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm followed by (61.35), (57.93) and (54.80) with the treatments of BAP 3.00ppm + IBA 0.50ppm, BAP 3.00ppm + IBA 1.50ppm and BAP 1.50ppm + IBA1.00ppm, respectively; while the minimum (37.06ppm) was noted under BAP 1.50ppm + IBA 0.50ppm.

Table: 5. Percentage of shoot development for pepino plantlets

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	37.06	61.35	52.54
IBA 1.00 ppm	54.80	67.79	48.57
IBA 1.50 ppm	40.29	57.93	51.47
Gen. Mean	44.05	62.36	50.86
C.D.	9.42	3.62	1.99
SE(m)	2.67	1.02	0.56
SE(d)	3.77	1.45	1.54
C.V.	10.50	2.85	3.66

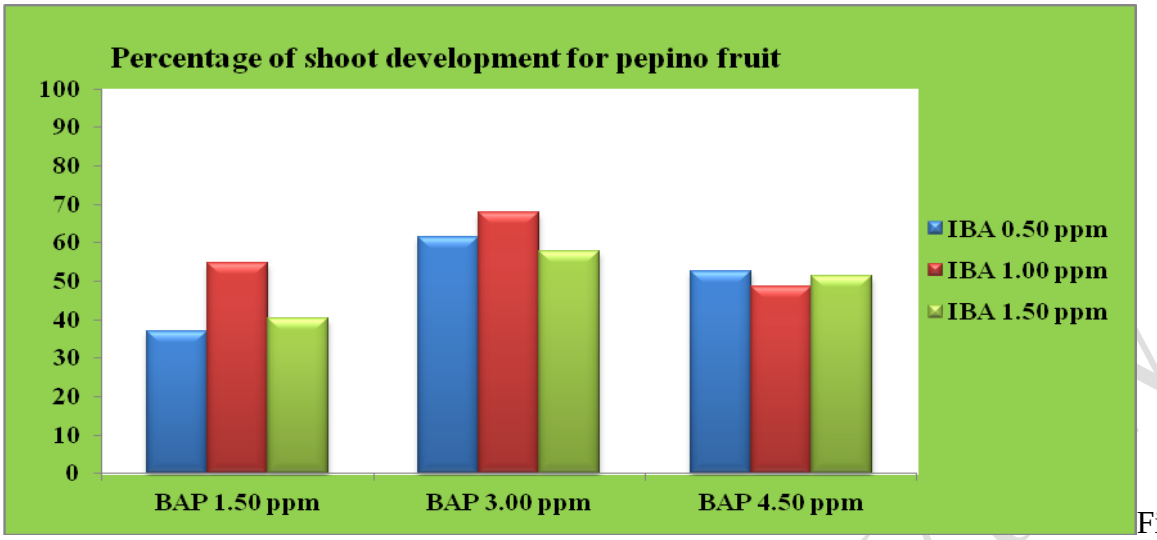

Fig. 4. Percentage of shoot development ofpepino plantlets

3.5. Number of shoots per explants/ inoculums of pepino plant

The data explain that number of shoots per explants/ inoculums from the established explants in the culture medium was significantly increased from 1.60 to 4.06 with the addition of BAP 1.50ppm + IBA 0.50ppm and BAP 3.00ppm + IBA 1.00ppm, respectively (Table no. 6 and Fig. no. 5). Maximum shoots per explants/ inoculums from the established culture (4.06) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm; followed by (3.67), (3.23) and (2.48) with the treatments of BAP 3.00ppm + IBA 1.50ppm, BAP 3.00ppm + IBA 0.50ppm and BAP 4.50ppm + IBA 1.00ppm respectively; while the minimum number of shoots per explants/ inoculums (1.60) was noted under BAP 1.50ppm + IBA 0.50ppm. A critical observation was recorded as the maximum number of shoots per explants/ inoculums in the culture (4.06) was recorded under the treatment of BAP 3.00 ppm + IBA 1.00ppm; while the minimum number of shoots per explants/ inoculums (1.50) was observed under BAP 1.50ppm + IBA 0.50ppm.**Table: 6. Number of shoots per explants/ inoculums of pepino plantlets**

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	1.60	3.23	2.16
IBA 1.00 ppm	2.11	4.06	2.48
IBA 1.50 ppm	2.05	3.67	2.24
Gen. Mean	1.92	3.65	2.29
C.D.	0.41	0.11	0.19
SE(m)	0.11	0.03	0.05
SE(d)	0.16	0.04	0.08
C.V.	10.62	1.57	4.24

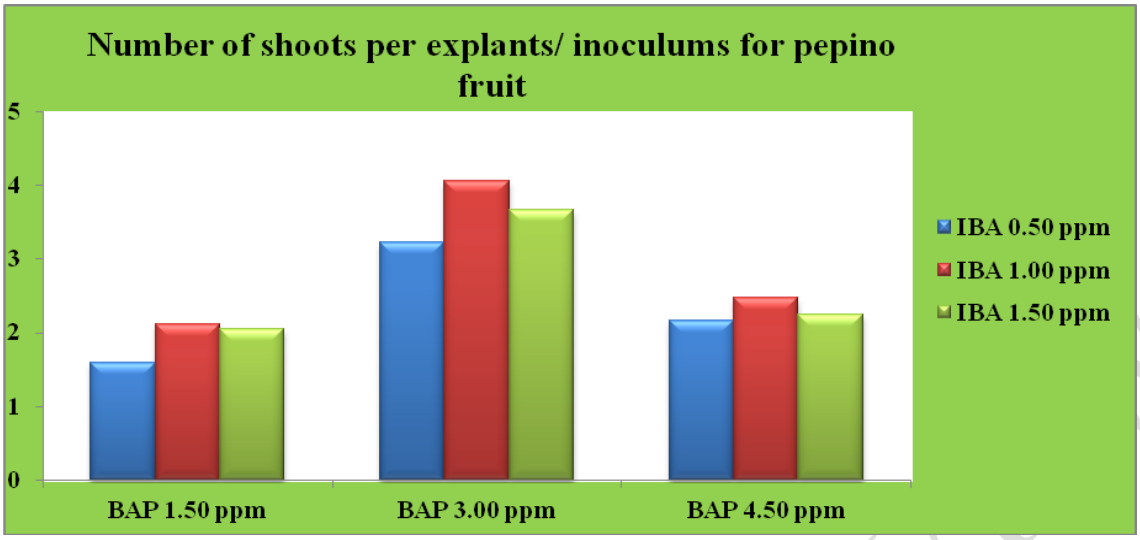

Fig. 5: Number of shoots per explants/ inoculums ofpepino plant

3.7. Number of leaves/plantlet of pepino plant

The data explain that optimum number of leaves/plantlet from the established explants in the culture medium was significantly increased from (5.14) to (10.85) with the addition of BAP 1.50ppm + IBA 0.50ppm and BAP 3.00ppm + IBA 1.00ppm respectively (table no. 7 and fig. no. 6). Maximum number of leaves / plantlet from the established culture (10.85) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm; followed by (8.73), (8.51) and (7.94) with the treatments of BAP 3.00ppm + IBA 1.50ppm, BAP 3.00ppm + IBA 0.50ppm and BAP 4.50ppm + IBA 1.00ppm respectively; while the minimum number of number of leaves / plantlet (5.37) was noted under BAP 1.50ppm + IBA 0.50ppm. A critical observation was recorded as the maximum number of number of leaves / plantlet in the culture (10.85) was recorded under the treatment of BAP 3.00 ppm + IBA 1.00ppm; while the minimum number of number of leaves / plantlet (5.37) was observed under BAP 1.50ppm + IBA 0.50ppm.

Table: 7. Number of leaves/plantletofpepino plantlets

Treatments	BAP 1.50ppm	BAP 3.00ppm	BAP 4.50ppm
IBA 0.50 ppm	5.14	8.51	6.43
IBA 1.00 ppm	7.41	10.85	7.94
IBA 1.50 ppm	5.37	8.73	6.61
Gen. Mean	5.97	9.36	6.99
C.D.	0.84	1.25	0.90
SE(m)	0.23	0.35	0.25
SE(d)	0.33	0.50	0.36
C.V.	6.93	6.56	6.34

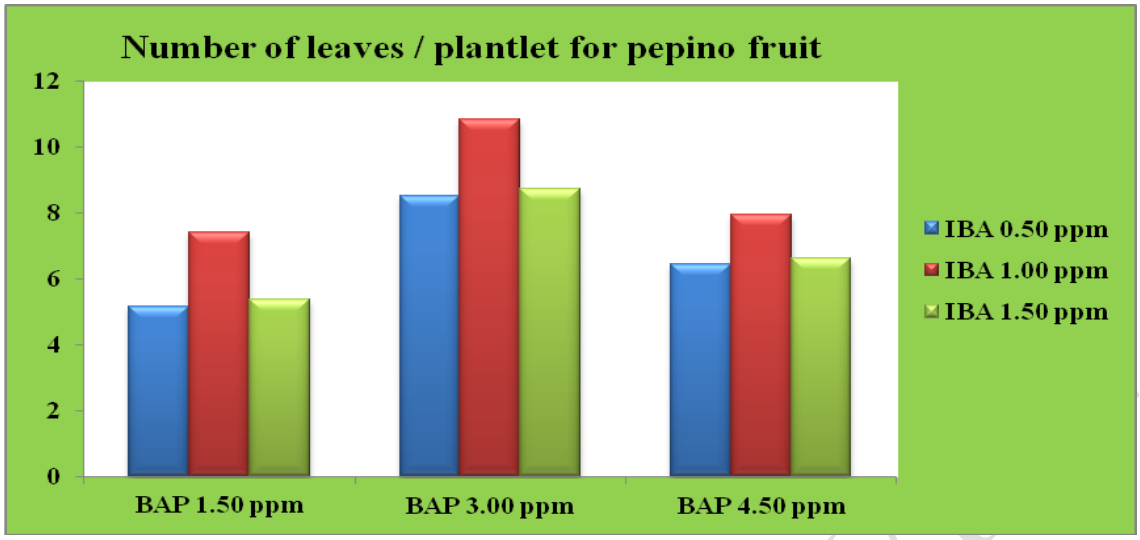

Fig. 6. Number of leaves / plantlet of pepino plantlets

4. DISCUSSION

4.1. Days taken for shoot initiation of pepino

The ratio of high cytokinin (BAP) and low auxin (IBA) to plays an important on in the Days taken for shoots initiation. Cytokinin alone has no effect on parenchyma cells. When cultured with auxin but no cytokinin, they grow large but do not divide. Explants cultured with cytokinin (BAP) and low auxin (IBA) resulted proper and early shoot initiation. A critical observation was recorded as the maximum days taken for shoot initiation in the culture (11.93 days) under the treatment of BAP 1.50 ppm + IBA 0.50ppm; while the minimum days taken for shoot development (5.27 days) was observed under BAP 3.00ppm + IBA 1.00ppm. This is consitant with the result obtained by(Faiz*yet al.*,2021),reported in 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 $^{-1}$ kinetin. The minimum days taken for shoot initiation 14.26 days with combination BAP 4.00 ppm and IBA 1.75ppm (Sedlak and Paprstein 2008),shoot initiation was achieved in sweet cherry cultivar Rivan micropropagated successfully using shoot culture medium with BAP. Addition of growth regulator BAP was essential for adventitious shoot initiation and development and(Diengngan*et al.*, 2016)excised the nodal segments of strawberry and cultured for 21 days on the initiation medium supplemented with various levels of BAP and TDZ. Minimum number of days to shoot initiation was obtained in MS medium supplemented with TDZ 0.5 and BAP 1.5 mg/l at 8.40±0.40 and 8.40±0.31 days respectively.

4.2. Days taken for shoot development of pepino

High concentration of cytokinins (BAP) and low concentration of auxin (IBA) are involved in many explants growth and development processes due to including cell division and shoot morphogenesis. They are known to regulate axillary bud growth and apical dominance. The direct inhibition hypothesis posits that these effects result from the cytokinin to auxin ratio. This theory states that auxin from apical buds travels down shoots to inhibit axillary bud growth. This promotes shoot growth, and restricts lateral branching. A critical observation was recorded as the maximum days taken for shoot development in the culture (52.54 days) under the treatment of BAP 4.50 ppm + IBA 0.50ppm; while the minimum days taken for shoot development (40.82 days) was observed under BAP 3.00ppm + IBA 1.00ppm. The result is similar with the findings of highest percentage of multiple shoot induction was achieved on MS + BAP (2.0 mg/l) + NAA (0.1 mg/l) by(Girijashankar*et al.*, 2011) in strawberry cv. Selva and they found maximum shoot development with combination, BAP+IBA different combination (Goswami *et al.*, 2013)developed a micropropagation protocol for multiplication of seedless lemon (*Citrus limon* L. cv. Kaghzi Kalan) using nodal explants for shoot development and they had reported decreased shoot development with increasing concentration of BAP alone, but in case of a combination of BAP and NAA (0.1 mg/l each)shoot development was highest in tissue culture protocol for in-vitro propagation of papaya (Podikunju*et al.,* 2017).

4.3. Length of shoot of plantlets (cm) of pepino

Cytokinin moves from the roots into the shoots and auxin from apical bud to roots eventually signaling lateral bud growth. When the apical bud is removed, the axillary buds are uninhibited, lateral growth increases, and plants become bushier cut stem again inhibits lateral dominance while cytokinin action in vascular plants is described as pleiotropic, induces the transition from apical growth to growth via a three-faced apical cell in moss protonema. This bud induction can be pinpointed to differentiation of a specific single cell, and thus is a very specific effect of high cytokinin (BAP) and low auxin (IBA) on length of shoot. The highest length of shoot (9.52 cm) was produced in media supplemented with BAP 3.00 ppm + IBA 1.00ppm and lowest length of shoot in (2.47 cm) belonged to media with BAP 1.50ppm + IBA 0.50ppm. The similar trend in length of shoot in (cm) was reported by also (Yeasmin *et al.,* 2022)and they had recorded high

length of shoot in strawberry that was with 0.5 mg/l BAP and 0.05 mg/l IBA showed highest shoot elongation efficiency(Singh *et al.,* 2007) and at proliferation stage, media supplemented with kinetin concentrations (4.7 µM and 9.2 µM) of along with (0.54 µM) NAA was used to be more efficient mediumresulting in the highest shoot length two Iranian commercial pomegranates by (ValizadehKaji*et al.,* 2013).

4.4 Percentage of shoot development of pepino plantlets

Maximum per cent of developed shoots obtained from the established culture (67.79 per cent) was noted under the treatment of BAP 3.00ppm+ IBA 1.00ppmfollowed by (61.35 per cent), (57.93 per cent) and (54.80 per cent) with the treatments of BAP 3.00ppm + IBA 0.50ppm, BAP 3.00ppm+ IBA 1.50ppmand BAP 1.50ppm+ IBA 1.00ppm, respectively; while the minimum (37.06 per cent) was noted under BAP 1.50ppm + IBA 0.50ppm. The finding was similar to the result of observed in mulberry that MS medium supplemented with 2mg/l BAP + IBA 1.50 2mg/l showed a maximum percentage (90%) of induced shoots (Roy *et al.*, 2012), reported maximum percentage (89%) *in-vitro* propagation of papayacv. Shahi through in vitro culture with combination of various kinds of plant growth hormones such as BAP concentrations in combination with NAA, Kinetin, IBA were preferred for use to initiate cultures(Kumari, 2023) recorded longest shoot in papaya variety CO-5 with application of BA 0.50 mg l^{-1} alone and with NAA 0.10 mg L^{-1} was found to be better for initial culture establishment and proliferation of papaya variety.

4.5. Number of shoots per explants/ inoculums of pepino plant

The ratio of high cytokinin (BAP) and low auxin (IBA) plays an important role in the effect of number of shoots per explant; more cytokinin induces growth of shoot buds, while less auxin support growth and development of shoot. Maximum shoots per explants/ inoculums from the established culture (4.06) was noted under the treatment of BAP 3.00ppm + IBA 1.00ppm and minimum number of shoots per explants/ inoculums (1.60) was noted under BAP 1.50ppm + IBA 0.50ppm. Similar observation was recorded on increasing in number of shoot per expalnts/ innoculam by(Diengngan*et al.*, 2016)they reported the maximum number of shoots per explants obtained in MS medium supplemented with 1mg/l TDZ at 6.40±0.37 and 3.50±0.07 cm, respectively in strawberry (Nguyen*et al.,* 2018) reported BAP in combination with NAA was

found to be the best in terms of number of shoot per explants that produced with medium supplemented with BAP (1.0 mgL-1) and NAA (0.1 mgL-1) in papaya as well as (Roy *et al.*, 2012) who reported higher shoot regeneration from lateral buds on media supplemented with BAP (0.5 mgL^{-1}) in combination with NAA (0.1 mgL^{-1}) compared to BAP or kinetin in papayaand (Jhajhra*et. al.*, 2018) found maximum number of shoots with MS medium + 1 ppm BA + 0.1 ppm NAA + 1 ppm adenine sulphate + 150 ml coconut water in Strawberry.

4.6. Number of leaves / plantlet of pepino plant

High concentration of cytokinins and low auxin are most abundant in growing tissues, such as leaves, shoot, roots, and cell division. Cytokinins are known to delay senescence in leaf tissues, promote mitosis, and stimulate differentiation of the meristem in shoots. Many effects on explant development are under the influence of cytokinin, either in conjunction with auxin. For example, apical dominance seems to result from a balance between auxin that inhibit lateral buds and cytokinin that promote bushier growth of leaves. The same pattern of performance of observedgreen color calli were pooled together and placed for adventitious shooting on MS medium supplemented with BAP 0.5 - 2 mg/l , NAA 0.1- 1 mg/l , Kinetin 0.5 - 1 mg/l, GA3 0.5 mg/l and Adenine sulphate 40 mg/l at different concentrations by (Guranna*et al.*, 2017) and recorded maximum number of leaves on medium containing MS + 2.5 mg/l BAP and (Saridas*et al.*, 2021), reported maximum number of leaves was obtained from MS medium containing 1 ppm BA, 0.1 ppm NAA, 1 ppm adenine sulfate and 150 mL coconut water together with maximum number of leaves shoots per explants in strawberry.

CONCLUSIONS

The effect of different concentrations of auxin as well as cytokinin were studied on shoot initiation, formation and multiplication with MS semi-solid mediumtwo different types of explants *viz.*, callus andinternodes.The growth hormone was taken alone and in combination of different concentrations of BAP and IBA. The MS media supplemented with BAP 3.00ppm+ IBA 1.00ppm gave the highest result for shoot initiation, shoot formation and multiplication of pepino fruit plant. This combination of plant growth hormone was recorded superior for all observation and treatment.

Fig 7. Shoot initiation and development

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