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EFFECT OF CYTOKININ AND AUXIN ON CALLUS FORMATION AND SHOOT MULTIPLICATION OF STRAWBERRY (*Fragaria* × *ananassa* Duch.) UNDER *IN VITRO* CONDITION

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ABSTRACT : The experiment was pursued in Tissue Culture Laboratory of Department of Horticulture in Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut during 2015-16 on Chandler variety of strawberry. N_6 media were prepared. Maximum callus formation in mature leaf explant (81%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0 mgl^{-1}. Maximum callus formation in young leaf (74.0%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0mgl^{-1}.Maximum callus induction in internode (47.6%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0 mgl^{-1} . Highest number of shoots (14.00) from mature derived callus at four weeks after inoculation were noted under the treatment of BAP 2mgl^{-1}+ Kinetin 1.5 mgl^{-1}. Maximum number of shoots (11.66) from young leaf derived callus of strawberry cv Chandler at four weeks after inoculation were noted under BAP 2mgl^{-1} combined with Kinetin 1.5 mgl^{-1} and BAP 3mgl^{-1} alone. The highest number of shoots (10.33) from internode derived callus at four weeks after inoculation were noted with BAP 3mgl^{-1} alone. Viewing above observations it is concluded that BAP 2 mgl^{-1} + IBA 1.5 mgl^{-1} and Kinetin 1.5 mgl^{-1} + IBA 1.0mgl^{-1} showed better performance on accordance of callus formation in mature leaf, young leaf as well as internode. BAP 2 mgl^{-1} + Kinetin 2mgl^{-1} showed better performance on accordance of shoot induction in mature leaf, young leaf as well as internode.

Keywords : *Strawberry, kinetin, cytokinin, auxin, callus, in-vitro.*

In tissue culture, cytokinins and auxins are important media components in determining the developmental pathways of the plant cells. Two major properties of cytokinins that are useful in culture are, stimulation of cell division together with auxins and release of lateral bud dormancy. They can also induce adventitious bud formation in cultures. The cytokinins stimulate cell division and the emergence of branches of callus tissue and organs and the development of buds by finishing the sovereignty of the apical buds.

Strawberry is very rich source of antioxidants. This fruit crop is grown in subtropical as well as temperate regions in India particularly in Punjab, Himachal Pradesh, Uttarakhand and Karnataka. However, the foot hills and Tarari regions are very much suited for its growth and development. So, western Uttar Pradesh may be well suited for its commercial cultivation.Viewing of above facts, the multiplication of disease free strawberry through tissue culture is needed for the production of numerous plants within a short period of time.

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MATERIALS AND METHODS

The experiment was conducted in Tissue Culture Laboratory of Department of Horticulture at Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut (U.P.), India during 2015-16. Chandler variety of strawberry was taken for experiment. N_6 media were prepared as per Cao and Hammerschlag (4). The stock solutions of BAP (Benzyl amino purine), Kinetin and IBA (Indole Butyric acid) were prepared and poured into the media treatments accordingly. All the stock solutions of auxin and cytokinin were stored in refrigerator after use.100 ml of stock A and 1 ml each of stock Solution B, C and 5ml stock solution D were taken in well sterilized reagent bottle of 1000ml capacity. Sucrose @15g per litre, agar powder as gelling agent @ 8 g per litre were added. Then, volume of 1000 ml was maintained through double distilled water. The pH5.85 was maintained either through 1N HCl or 1N NaOH. From BAP stock solution of 1000 ppm: 1ml, 2ml and 3ml, similarly from Kinetin stock solution of 1000 ppm 1ml, 1.5 ml and 2 ml as from IBA (Indole Butyric acid) of 1000 ppm 0.5 ml, 1.0 ml and 1.5 ml were added seperately into the

above solution accordingly on the basis of different treatments. The specific solutions were boiled under water bath just to dissolve the whole ingradients well. Then they were cooled down and 20 ml each solution was poured separately into sterilized culture containers and they were closed with lid. Then the whole culture containers filled with media were autoclaved with 121.6°C at 15 psi for 20 minutes. Mature, young leaves and internodes of chandler had been used as explants to produce callus. The leaves (mature and young) and internode of strawberry were collected, kept in non-dehydrating conditions until sterilization, *e.g.* in a container with a few drops of water and sealed with a plastic film. These explants were thoroughly washed with double distilled water. Then they were kept in 70 per cent ethyl alcohol for a period of 5 minutes. After that they were washed with double distilled water stirring well. This process was applied five times just to wash out the adhering chemicals on the surface of the explants. The whole explants were kept in the sterilized container under Laminar Flow.The sterilized explants were put on the floor of the Laminar Flow and they were gently surrounded by few drops of ethyl alcohol through smearing with sterilized cotton before opening of front lid. In this way whole of explants were thoroughly surface sterilized. Then the lower and upper portion of explants was cut and removed through sterilized knife and placed as "Initial explant" in a callus induction medium to develop into an embryogenic callus under the flame spirit lamp to keep the surrounding free from microorganisms. Then the inoculated culture containers were sealed with paraffin film to make the container free from germs. The inoculated containers were placed into the Growth Room for initiation of callus under 25ºC and 60 per cent RH. Timely observation was taken and contaminated containers were picked up and kept out of the Growth Room. The Two way Factorial design was used for statistical analysis.

RESULTS AND DISCUSSION

Callus formation percentage:

Significant increase in average callus formation percentage in mature leaves were recorded from 54.6% to 81.0% at four weeks after inoculation.The maximum callus formation (73.9%) was observed in treatment BAP 2mgl^{-1} while the minimum (57.8%) was noted under BAP 2mgl. The maximum callus induction percentage (70.00) was noted under the treatment IBA 1.0 mg^{-1} incorporated the medium while the minimum was observed under IBA 0.5 mgl^{-1} (Table 1).

Table 1: Effect of different concentrations of BAP and Kinetin with IBA on calli formation of manure leaf of strawberry cv Chandler at four weeks after inoculation.

Treatments	Callus induction: mature leaf			
	IBA 0.5	IBA 1.0	IBA 1.5	Mean
BAP 1	61.66	77.33	66.67	68.6
BAP 2	68.33	81.00	72.33	73.9
BAP 3	54.67	60.00	58.67	57.8
Kinetin 1.0	61.00	65.33	65.67	64.0
Kinetin 1.5	70.67	70.67	67.67	69.7
Kinetin 2.0	61.33	65.67	58.33	61.8
Mean	62.94	70.00	64.89	

C.D. (P=0.05): Main Effect: = 2.24; IBA = 1.58; Interaction= 3.88

Table 2 : Effect of different concentrations of BAP and Kinetin with IBA on calli formation of young leaf of strawberry cv Chandler at four weeks after inoculation.

Treatments	Callus induction : Young leaf			
	IBA0.5	IBA 1.0	IBA 1.5	Mean
BAP 1	55.33	72.00	63.00	63.4
BAP 2	63.33	74.00	64.67	67.3
BAP 3	48.33	56.00	52.33	52.2
Kinetin 1.0	56.00	56.67	58.00	56.9
Kinetin 1.5	54.00	63.33	63.00	60.1
Kinetin 2.0	52.00	57.00	51.67	53.6
Mean	54.83	63.17	58.78	

C.D. (P=0.05): Main Effect = 2.43; IBA = 1.72; Interaction = 4.22

Significant increase in average callus formation percentage in young leaves were recorded from 48.33% to 74.00% at four weeks after inoculation. Maximum callus formation (67.3%) was observed in treatment BAP 2mgl^{-1} followed by 63.4, 60.1 and and 56.9 per cent while the minimum (52.2%) was noted under BAP 3mgl^{-1}. The maximum callus induction percentage (57.00) was noted under the treatment IBA 1.0mg^{-1}incorporated the medium while the minimum was observed under IBA 0.5mgl^{-1}.

Table 3: Effect of different concentrations of BAP and Kinetin with IBA on calli formation of internode of strawberry cv Chandler at four weeks after inoculation

Treatments	Callus induction: Internode			
	IBA0.5	IBA 1.0	IBA 1.5	Mean
BAP 1	32.33	42.33	35.00	36.6
BAP 2	35.67	47.67	33.00	38.8
BAP 3	28.00	27.33	33.00	29.4
Kinetin 1.0	36.67	34.67	33.67	35.0
Kinetin 1.5	33.00	33.00	31.33	32.4
Kinetin 2.0	33.00	35.67	31.00	33.2
Mean	33.11	36.78	32.83	

C.D. (P=0.05): Main Effect: = 2.96; IBA= 2.09; Interaction = 5.13

The callus formation percentage in internode was significantly increased from 27.33% to 47.67% at four weeks after inoculation.Significantly maximum callus formation (38.8%) was observed in treatment BAP 2 mgl^{-1} followed by 36.6, 35.0 and 33.2 per cent while the minimum (29.4%) was noted under BAP 3 mgl^{-1}. The maximum callus induction percentage (36.7%) was noted under the treatment IBA 1.0 mg^{-1} incorporated the medium while the minimum was observed under IBA 0.5mgl^{-1} .A minute observation was recorded as the maximum callus induction (47.6%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0 mgl^{-1} followed byBAP 1mgl-1combined with IBA 1.0mgl^{-1} , Kinetin 1 mgl^{-1} with IBA 0.5mgl^{-1}, BAP 2mgl^{-1} with IBA 0.5 mgl^{-1} and BAP 1.0 mgl^{-1} with IBA 1.5mgl^{-1}; however, they were significantly at par with each other. The minimum (48.3%) was recorded under BAP 3mgl^{-1} with IBA 1.0mgl^{-1}.

The callus formation percentage was recorded among the different treatments of BAP (Benzyl amino purine), Kinetin, IBA (Indole butyric acid) and their different combinations at different periods. Incorporation of BAP from 1 to 3 mgl^{-1}, Kinetin1 to 2 mgl^{-1} and IBA 0.5 to 1.5 mgl^{-1} and their different combinations in culture medium showed great variability among the treatments under study.

The treatments under study for a period of four weeks showed that significant the maximum callus formation(81%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0mgl^{-1} followed byBAP 1mgl^{-1} combined with IBA 1.0mgl^{-1}, BAP 2mgl^{-1} with IBA 1.5 mgl^{-1} , Kinetin 1.5 mgl^{-1} with IBA 0.5 mgl^{-1} and Kinetin 1.5 mgl^{-1} with IBA 1.0mgl^{-1} . while the minimum (54.6%) was recorded under BAP 3mgl^{-1} with IBA 0.5 mgl^{-1} . The same trend of observation was noted under BAP and BA treatments alone. It showed that as the concentration of BAP and Kinetin icreases from 1 to 2 and 1 to 1.5mgl^{-1} separately combined with IBA @1.0mgl^{-1} increases the percentage of callus initiation under aseptic condition. However, an increase in the concentrations of growth regulators cause decreasing effects. Further, it was observed that mature leaf did better result in response to callus formation. This might be due to balanced content of cytokinin and auxin level in the mature leaf as compared to young leaf and internode.

Maximum callus induction in young leaf (74.0%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0 mgl^{-1} followed by BAP 1 mgl^{-1} combined with IBA 1.0 mgl^{-1} , BAP 2mgl^{-1} with IBA 1.5 mgl^{-1} , BAP 2 mgl^{-1} with IBA 0.5mgl^{-1} and Kinetin 1.5 mgl^{-1} with IBA 1.0 mgl^{-1} while the minimum (48.3%) was recorded under BAP 3mgl^{-1} with IBA 0.5 mgl^{-1}.

Maximum callus induction in internode (47.6%) was noted under the treatment of BAP 2 mgl^{-1} combined with IBA 1.0 mgl^{-1} followed by BAP 1 mgl^{-1} combined with IBA 1.0 mgl^{-1} , Kinetin 1 mgl^{-1} with IBA 0.5 mgl^{-1}, BAP 2 mgl^{-1} with IBA 0.5 mgl^{-1} and BAP 1.0 mgl^{-1} with IBA 1.5 mgl^{-1} ; however, they were significantly at par with each other. The minimum (48.3%) was recorded under BAP 3 mgl^{-1} with IBA 1.0mgl^{-1} . The same pattern of observations were recorded by Owen *et al.* (14), Sutter *et al.* (16), Wawrzynczak *et al.* (17), Biswas *et al.* (3), Moradi *et al.* (10), Ara *et al.* (1) and Ashrafuzzaman *et al.* (2).

Shoot induction

Table 4 : Effect of different concentrations and combinations of growth regulators for shoot induction from mature leaf derived callus of strawberry cv Chandler at 21 days after inoculation.

Treatments	Shoot Induction % : Mature leaf				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	7.67	28.67	34.00	32.67	25.8
BAP 1	29.00	36.67	41.33	55.00	40.5
BAP 2	49.33	54.67	58.33	62.33	56.2

BAP 3	33.00	46.33	49.00	54.00	47.1
Mean	31.25	41.58	45.67	51.00	

C.D. (P=0.05): BAP: = 3.12; Kinetin= 3.12; Interaction = 6.25

Average shoot induction percentage from mature leaf derived callus of strawberry cv. Chandler was significantly increased from 7.66% to 62.33% at 21 days after inoculation. Significantly maximum shoot induction 56.2 per cent was noted from mature leaf derived callus of strawberry cv Chandler was observed in treatment BAP 2mgl^{-1} followed by 47.1 and 40.5 per cent while the minimum (25.8%) was noted under no addition of BAP in the medium. The maximum shoot induction percentage (51.00%) was noted under the treatment Kinetin 2.0 mgl^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.A minute observation was recorded as the maximum shoot induction (62.33%) was noted under the treatment of BAP 2mgl^{-1} combined with Kinetin 2.0mgl^{-1} followed byBAP 2mgl^{-1} with Kinetin 1.5mgl^{-1}, BAP 1mgl^{-1} with Kinetin 2.0mgl^{-1}, BAP 2mgl^{-1} with Kinetin 1.0mgl^{-1} and BAP 3.0 mgl^{-1} with Kinetin 2.0mgl^{-1} however, they were significantly at par with each other. The minimum (7.66%) was recorded under control.

Table 5: Effect of different concentrations and combinations of growth regulators for shoot induction from young leaf derived callus of strawberry cv Chandler at 21 days after inoculation.

Treatments	Shoot Induction % : Young leaf				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	5.33	23.00	27.67	30.00	21.50
BAP 1	26.00	29.00	31.00	44.67	32.67
BAP 2	36.33	52.33	51.33	57.00	49.25
BAP 3	34.67	42.33	43.67	45.67	41.58
Mean	25.58	36.67	38.42	44.33	

C.D. (P=0.05): BAP = 2.47; Kinetin: = 2.47; Interaction= 4.94

The average shoot induction from young leaf derived callus of strawberry cv Chandler was significantly increased from 5.33% to 57.00% at 21 days after inoculation.The treatments under study showed that significantly maximum shoot induction 49.2 per cent was noted from young leaf derived callus of strawberry cv. Chandler was observed in treatment BAP 2mgl^{-1} followed by 41.5 and 32.6 per cent while the minimum (21.5%) was noted under no addition of BAP in the medium. The highest shoot induction percentage (44.33%) was noted under the treatment Kinetin 2.0mg^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.The maximum shoot induction (57.0%) was noted under the treatment of BAP 2mgl^{-1}combined with Kinetin 2.0mgl^{-1} followed byBAP 2mgl^{-1}with Kinetin 1.0mgl^{-1} and BAP 2mgl^{-1} with Kinetin 1.5mgl $^{-1}$; however, they were significantly at par with each other. The minimum (5.33%) was recorded under control.

Table 6 : Effect of different concentrations and combinations of growth regulators for shoot induction from internode derived callus of strawberry cv Chandler at 21 days after inoculation.

Treatments	Shoot Induction % : Internode				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	6.00	29.33	33.33	36.33	26.3
BAP 1	22.33	36.67	40.00	42.67	35.4
BAP 2	37.33	42.00	45.67	47.67	43.2
BAP 3	33.00	39.67	40.00	43.00	38.9
Mean	24.67	36.92	39.75	42.42	

C.D. (P=0.05): BAP = 2.38; Kinetin= 2.38; Interaction= 4.76

The Table 6 shows that average shoot induction percentage from internode derived callus of strawberry cv Chandler was significantly increased from 6.00% to 47.66% at 21 days after inoculation.The treatments under study showed that significantly maximum shoot induction 43.2 per cent was noted from internode derived callus of strawberry cv Chandler was observed in treatment BAP 2mgl^{-1} followed by 38.9 and 35.4 per cent while the minimum (26.3%) was noted under no addition of BAP in the medium. The highest shoot induction percentage (42.41%) was noted under the treatment Kinetin 2.0mg^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.A minute observation was recorded as the maximum shoot induction (57.0%) was noted under the treatment of BAP 2mgl-1combined with Kinetin 2.0mgl $^{-1}$ followed by BAP 2mgl^{-1} with Kinetin 1.5mgl^{-1}, BAP 3mgl^{-1} with Kinetin 2mgl^{-1} and BAP 1mgl^{-1} with Kinetin 2mgl^{-1} however, they were significantly at par with each other. The minimum (6.00%) was recorded under control.

Shoot multiplication

Table 7 : Effect of different concentrations and combinations of growth regulators for shoot multiplication from matureleaf derived callus of strawberry cv. Chandler at four weeks after inoculation.

Treatments	Number of shoots after 4 weeks				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	3.00	7.00	8.67	12.00	7.67
BAP 1	7.67	10.00	10.33	12.00	10.00
BAP 2	10.67	13.00	14.00	13.00	12.67
BAP 3	13.67	13.00	13.00	12.00	12.02
Mean	8.75	10.75	11.50	12.25	

C.D. (P=0.05): BAP = 1.09; Kinetin= 1.09; Interaction= 2.18

The Table 7 evinces that average number of shoots from mature leaf derived callus of strawberry cv Chandler was significantly increased from 3.00 to 14.00 at four weeks after inoculation. Significantly maximum number of shoots (12.67) were noted from mature leaf derived callus of strawberry cv Chandler was observed in treatment BAP 2mgl^{-1} followed by 12.02 and 10.00 while the minimum (7.6) was noted under no addition of BAP in the medium. The highest number of shoots (12.25) were noted under the treatment Kinetin 2.0mgl^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.A minute observation was recorded as the maximum number of shoots (14.00) were noted under the treatment of BAP 2mgl^{-1} combined with Kinetin 1.5mgl^{-1} followed byBAP 3mgl^{-1} alone, BAP 2.0mgl^{-1} with Kinetin 1 & 2.0 mgl^{-1} and BAP 3mgl^{-1} with Kinetin 1 & 1.5mgl^{-1}; however, they were significantly at par with each other. The minimumshoots (3.00) were recorded under control.

Table 8 : Effect of different concentrations and combinations of growth regulators for shootmultiplication from young leaf derived callus of strawberry cv Chandler at four weeks after inoculation

Treatments	Number of shoots after 4 weeks				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	2.67	4.33	6.00	10.00	5.75
BAP 1	6.00	9.00	9.33	10.67	8.75
BAP 2	8.67	10.00	11.67	11.00	10.33
BAP 3	11.67	10.00	11.00	10.00	10.67
Mean	7.25	8.33	9.50	10.42	

C.D. (P=0.05): BAP: = 0.93; Kinetin= 0.93; Interaction= 1.87

The data evince that average number of shoots from young leaf derived callus of strawberry cv Chandler was significantly increased from 2.66 to 11.66 at four weeks after inoculation.The maximum number of shoots (10.67) were noted from young leaf derived callus of strawberry cv Chandler was observed in treatment BAP 3mgl^{-1} followed by 10.33 and 8.75 while the minimum (5.75) was noted under no addition of BAP in the medium. The highest shoots (10.41) were noted under the treatment Kinetin 2.0mg^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.

Table 9 : Effect of different concentrations and combinations of growth regulators for shoot multiplication from internode derived callus of strawberry cv Chandler at four weeks after inoculation.

Treatments	Number of shoots after 4 weeks				
	Kinetin 0	Kinetin 1	Kinetin 1.5	Kinetin 2	Mean
BAP 0	2.67	3.67	5.33	8.67	5.08
BAP 1	5.33	8.33	9.33	9.67	8.17
BAP 2	7.33	9.33	10.00	10.00	9.17
BAP 3	10.33	9.00	9.67	9.67	9.67
Mean	6.42	7.58	8.58	9.50	

C.D. (P=0.05): BAP: = 1.02; Kinetin= 1.02; Interaction= 2.05

The data evince that average number of shoots from internode derived callus of strawberry cv Chandler was significantly increased from 2.66 to 10.33 at four weeks after inoculation.The maximum number of shoots (9.67) were noted from internode derived callus of strawberry cv Chandler was observed in treatment BAP 3mgl^{-1} followed by 9.17 and 8.17 while the minimum (5.08) was noted under no addition of BAP in the medium. The maximum shoots (9.50) were noted under the treatment Kinetin 2.0mgl^{-1} incorporated the medium while the minimum was observed under no addition of Kinetin.A minute observation was recorded as the maximum number of shoots (10.33) were noted under the treatment of BAP 3mgl^{-1} alone followed byBAP 2mgl^{-1} with Kinetin 1.5 & 2.0mgl^{-1}, BAP 3.0mgl^{-1} combined with Kinetin 1.5 and 2.0mgl^{-1}; however, they were significantly at par with each other. The minimum shoots (2.66) were recorded under control.

Incorporation of BAP from 1 to 3 (mgl^{-1}) and Kinetin from 1 to 2 (mgl^{-1}) and their combinations in

culture medium were found great variability on shoot multiplication after callus formation.

Highest number of shoots (14.00) from mature derived callus of strawberry cv Chandler at four weeks after inoculation were noted under the treatment of BAP 2 mgl^{-1} combined with Kinetin 1.5mgl^{-1} followed by BAP 3 mgl^{-1} alone, BAP 2.0 mgl^{-1} with Kinetin 1 & 2.0 mgl^{-1} and BAP 3mgl^{-1} with Kinetin 1 & 1.5mgl^{-1} ; however, they were significantly at par with each other. The minimum shoots (3.00) were recorded under control.

Maximum shoots (22.66) at five weeks after inoculation were noted under the treatment of BAP 3mgl-1combined with Kinetin 2mgl^{-1} followed byBAP 3mgl^{-1} with Kinetin 1.5 & 1.0mgl^{-1} ; however, they were significantly at par with each other. The minimum shoots (5.66) were recorded under control.

Highest shoots (26.66) at six weeks after inoculation were noted under the treatment of BAP 3mgl^{-1} combined with Kinetin 2mgl^{-1} followed byBAP 3mgl^{-1} with Kinetin 1.5mgl, BAP 2mgl^{-1} combined with Kinetin 2mgl^{-1} and BAP 2mgl^{-1} combined with Kinetin 1.5mgl^{-1} however, they were significantly at par with each other. The minimum shoots (7.33) were recorded under control. As the time increases the number of shoots increases. So, there is positive correlation with time to multiplication of shoots. The same pattern of observations were recorded by Sorvar *et al.* (15), Owen *et al.* (14), Sutter *et al.* (16), Wawrzynczak *et al.* (17) Biswas *et al.* (3), Moradi *et al.,* (10), Ara *et al.,* (1) and Ashrafuzzaman *et al.* (2), Diengngan *et al.* (5) and Harugade *et al.* (7).

Maximum shoots (11.66) from young leaf derived callus of strawberry cv Chandler at four weeks after inoculation were noted under the treatment of BAP 2mgl-1combined with Kinetin 1.5mgl^{-1} and BAP 3mgl-1alone followed byBAP 2mgl^{-1} with Kinetin 2mgl, BAP 3mgl^{-1} combined with Kinetin 1.5mgl^{-1} and BAP 1mgl^{-1} combined with Kinetin 2.0mgl^{-1} however, they were significantly at par with each other. The minimum shoots (7.66) were recorded under control.

Effect of various concentrations of BAP, Kinetin and IBA on explant development: Explants that survived and exhibited some degree of growth were maintained and used for further studies. When cultures were shifted to the medium containing different combination of BAP alone, there was a significant increase in the mean number of shoot after 30 days of culture in N_6 medium containing 1-3 mgl^{-1} BAP, 1-2 mgl^{-1} Kinetin and 0.5-1.5 mgl^{-1} IBA.

There was a large degree of variation in the growth of plants cultured on different cultured media. All the explants did not show uniform rate of growth in terms time of callus formation, time of shoot initiation and number of shoots.

The highest number of shoots (10.33) from internode derived callus of strawberry cv Chandler at four weeks after inoculation were noted under the treatment of BAP 3mgl^{-1} alone followed by BAP 2mgl $^{-1}$ Kinetin 1.5 & 2.0mgl^{-1}, BAP 3.0mgl^{-1} combined with Kinetin 1.5 & 2.0mgl^{-1}; however, they were significantly at par with each other. The minimumshoots (2.66) were recorded under control.

The results showed that maximum number of shoots from the callus were emerged from mature leaf derived callus followed by young leaf and internode. This might be due to that the mature leaf is having more content of cytokinin which initiates the multiplication rate of shoots. The same pattern of observations were recorded by Nehra and Stushnoff (12), Nehra *et al.* (11), Greene *et al.* (6), Infante *et al.* (8), Cao and Hammerschlag (4), Ara *et al.* (1), Mahmoud and Kosar (9), Ashrafuzzaman *et al.* (2), Diengngan *et al.* (5), Harugade *et al.* (7) and Nikolic *et al.* (13).

CONCLUSION

Maximum callus induction in mature leaf (81.0%) as well in young leaf (74.0%) was noted under the treatment of BAP 2mgl^{-1}combined with IBA 1.0mgl^{-1} while the minimum (54.3%) was recorded under BAP 3mgl^{-1} with IBA 0.5mgl^{-1}.

Maximum callus induction in internode (47.6%) was noted under the treatment of BAP 2mgl^{-1} combined with IBA 1.0mgl^{-1}.

Highest number of shoots (14.00) from mature derived callus of strawberry cv. Chandler at four weeks after inoculation were noted under the treatment of BAP 2mgl^{-1} combined with Kinetin 1.5mgl^{-1}.

Maximum number of shoots (11.66) from young leaf derived callus of strawberry cv Chandler at four weeks after inoculation were noted under the treatment of BAP 2mgl^{-1}.

The highest number of shoots (10.33) from internode derived callus of strawberry cv. Chandler at four weeks after inoculation were noted under the

treatment of BAP 3mgl-1alone followed byBAP 2mgl^{-1} with Kinetin 1.5 & 2.0mgl^{-1}, BAP 3.0mgl^{-1} combined with Kinetin 1.5 & 2.0mgl^{-1}.

Viewing above observations it is concluded that BAP 2 mgl^{-1} + IBA 1.5mgl^{-1} and Kinetin 1.5 mgl^{-1} + IBA 1.0mgl^{-1} showed better performance on accordance of callus formation in mature leaf, young leaf as well as internode.BAP 2 mgl^{-1} + Kinetin 2mgl^{-1} showed better performance on accordance of shoot induction in mature leaf, young leaf as well as internode.

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