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RAPID MULTIPICATION OF STRAWBERRY (*Fragaria x ananassa* Dutch) BY *IN VITRO* RUNNER TIPS CULTURE

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ABSTRACT

A micropropagation procedure for rapid multiplication of strawberry by runner tips is described. The highest regeneration rate and most shoots per runner tips explants resulted on a medium based on Knop medium and M.S. micro element supplemented with (1mg/l) BAP and 0.5 mg/l kn together with glucose at a concentration of 4%. Glucose was more effective than sucrose or a combination of these sugars. Best results on rooting were obtained with Knop salt formulation having 1mg/l IBA. Addition of 300mg/l activated charcoal also enhanced rooting using this produce, 90%. of shoots, rooted. The survival rate at the end of the acclimatization period was 80%.

Key words: Knop medium, M.S. medium, Glucose, sucrose, Activated charcoal.

INTRODUCTION:

The cultivated strawberry *Fragaria* X *ananassa* Dutch is a vegetative propagated octoploid species (2n= 8x = 56) derived from *F. virginiana* and *F. chiloensis*. It is charaterized by high level of hetrozygosity mainly due to its hybrid background. Strawberry cultivars are well adapted to different climate (Mitra, 1991) like moderate mediterranean, subtropical and tropical. The use of micro propagation for producing pathogen free strawberry mother plants has been quite successful (Boxes1974, 1992, Marcotrigiano *et al.* 1984; Swartz and Lindstrom 1986). This investigating was undertaken to optimize *in vitro* culture condition for multiplying the strawberry c.v. Gorilla.

MATERIAL AND METHODS

Cultures were initiated from 1-2 long runner tips of virus free plants c.v. Gorilla. Explants were surface sterilized with sodium hypochlorite (5%) for 8 minutes followed by Tween-20 for half an hour. The runner tips were further rinsed with 70% ethanol (1min) followed by 0.1% $HgCl_2$ (5 min) and rinsed three times with sterile water. The culture medium used was modified Murashige and Skoog (1962), B5 (1968) and Knop (1965). Modification refer only to macro elements, since MS micro elements were used in all cases. Different types and concentration of sugars were added, supplemented with BAP, Kn singly on in combination. The pH was adjusted to 5.7 and the medium was autoclaved for20 min at 121^0c (0.1mPa). After culturing explants were incubated in a growth chamber at $23^0 \pm 1^0$C and 16hr, photoperiod at 3000 lux approximately. Single axillary shoot, over 5mm long were used in all experiment for sub culturing into new media every 4-5 weeks intervals, Glass growth tubes each containing 20ml of solid media were used for the initial phase of culture and for subsequent proliferation phase glass bottles containing 50ml of medium were used.

RESULTS

SHOOT INDUCTION: Runner segment were cultured in Knop medium and MS microelements with different concentration of BAP and Kinetin i.e. 0.0 mg/l (control), 0.5mg/l, 1.5mg/l, 2mg/l, (Table-1) after four weeks, the cultured explants were subcultured.

(Table-1) **Response of strawberry explants to different BAP and kinetin concentration supplemented in Knop macroelements and MS microelements having 4% Glucose.**

BAP mg/l	Kinetin mg/l	No of explants/culture	Average no of shoots/culture	Average length shoots/culture (cm)	Average no of leaves/culture
0.0	0.0	20	-	-	-
0.5	0.5	20	4	2	5
1.0	0.5	20	10	5	15
1.5	0.5	20	6	2.5	8
2.0	0.5	20	0.0	0.0	0.0

The highest average number of shoots (10), length of shoot (5 cm), Number of leaves 15 were observed of the concentration of 1.0 mg/l BAP and 0.5 mg/l kn, (Table1), (FigB), No shoots was induced of the high (2.0 mg/l) BAP and (0.5 mg/l kn). Rest of the treatment produced shoots that were fragile. The lowest average length (2cm) of shoots and average number (5) of leaves were observed in 0.5 mg/l BAP and 0.5 mg/l kn.

ROOT INDUCTION:

After five weeks the elongated shoots were transformed into the rooting media. Among the concentration IBA @ mg/l showed the best performance in all the parameters studied. (Table-2)

Table-2 Effects at different IBA concentration in Knop salt formulation on root induction ability of strawberry.

Treatment mg/l	Day to root initiation	Average no of root/culture	Average length of root/culture
0.0.	-	-	-
0.5	8-9	4	2.1
1.0	7-8	8	4.05
1.5	12-13	3	3.0
2.0	14-15	2	1.5
2.5	16-17	2	1.0

Only 1/mg/l IBA concentration took short as time (7-8 days) for root induction. The highest no (8) of root/culture and the longest (4.05cm) roots were also obtained from same concentration. On the other hand root produced by other treatment were narrow. No roots were produced in control treatment. Addition of 300 mg/l of activities charcoal also enhanced rooting in /mg/l IBA. (fig C).

DISCUSSION

In vitro culture of strawberry greatly affected the quality of material obtained and its field performance (Boxus 1974**);** Macrotrigiana *et al.* (1984); Rancillae *et al.* (1987); Zimmerman (1991). In c.v. 'Gorilla' use of 1-2 mm long runner tips allowed the establishment of *in vitro* culture with a 75-100% survival during March until May while contamination problem occurred with culture initiated during rest of year. The different salt formulation used significantly affected average shoot length of cultivar 'Gorilla' with Knop (1965) being better than MS (1962) or B5 (1968). However Ara *et al.* (2012) found MS medium, better than any other medium. Karim *et al.* (2015), Bhatt and Dhar (2000) reporting sprouting in strawberry using MS medium containing kinetin or in combination with BAP and NAA. In c.v. 'Gorilla' best results were obtained with Knop medium 10-15 auxiliary shoots per culture (5 subculture average). Proliferation rate decreased through sub culturing in MS or B5 formulation all shoots in MS were vitrified while those in B5 grew poorly but looked normal. In Knop medium shoots were of better quality.

MS, a high ionic strength salt formulation has been widely recommended for strawberry micropropagation by Beech *et al.* (1988), although our best result for c.v. Gorilla were obtained in Knop + MS minor. This result agree with several reports Shoemaker *et al.* (1985)

who pointed out that small fruits, particularly *Fragaria* X *ananassa* were sensitive to high nutrient levels in the culture medium.

The effects of different types and concentration of sugar on adventitious shoot regeneration was studied. When sucrose was replaced by glucose the percentage of regeneration increased.

Benzyladenine seems to be important in controlling proliferation of strawberry shoots. Macrotrigiana *et al.* (1984) observed on a large number of strawberries cultivars that BAP concentration in range of 1.3-13.3 μ, did not produce significant difference in multiplication rate in contrast Simpson and Bell (1989) found clear differences among genotypes in their BAP requirements for optimum shoot proliferation. The use of low BAP concentration on strawberry micropropagation has been recommended by Rancillac *et al*. (1987) since it decrease the risk of phenotypic abnormalities after the fields establishment of micropropagated plants, Mancotrigians and Swartz (1984).

In our case BAP concentration markedly affected c.v. 'Gorilla'. shoot *in vitro* generally thinner shoots were obtained when BAP was used alone (Fig A). when BAP was used along with kn shoots were of good quality (Fig B).

The addition of 300mg/l activated charcoal markedly affected rooting, roots appeared after a few days in the presence of activated charcoal (Fig C). The beneficial effects of activated charcoal has also been observed in the rooting of *Quercus rober,* Favre and Jancker (1987) and could be explained by its role in observing inhibitors eg exudates (Weatherhead *et al.* (1978)).

Best rooting of the shoots was obtained in 1mg/l IBA (Table 2). Buxus (1992) also obtained rooting by inclusion of IBA in the medium. Another factor affecting root quality was salt formulation best results were obtained at low to medium ionic strength eg Knop medium. High concentration of macro elements probably interface with root differentiation. Nemeth (1980) and Pleigo-alfaro (1988) have also shown the convenience of low ionic strength formulations to improve rooting, using this produced 90% of shoots rooted.

CONCLUSION

Belkengrean and Miller (1962) were first to recommended the use of meristem culture for elimination of viruses from *Fragaria.* Other workers have since used meristem tip culture to eliminate viruses from many commercial strawberry cultivars. Adams (1972) was the first report on micro propagation of strawberry. He concluded it would seem to be possible to obtain an unlimited number of plantlets from a single meristem. Nishi and Oasawr (1973) and Boxus (1974) have reported mass propagation of virus free strawberry plants. In our case for shoot induction Knop medium and MS minor elements gave the best results in presence of both BAP and kinetin hormone, for rooting IBA with charcoal gave best results. In this

investigation, a micro propagation protocol for strawberry has been developed. On the hand, this finding could be utilized in plant genetic transformation studies for better improvement.

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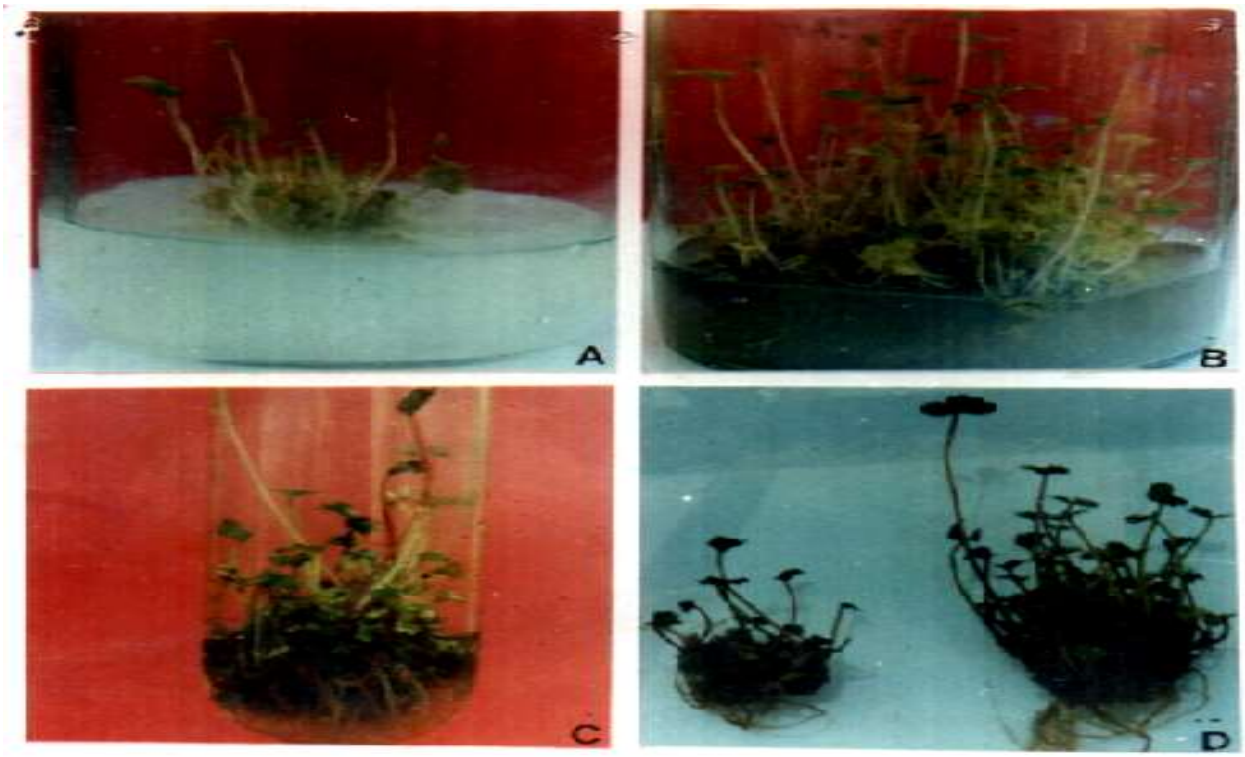

Fig: A. Thinner shoots when BAP used alone.
Fig: B. Good quality shoots when BAP used along with kinetin.
Fig: C. Rooting in presence of activated charcoal and IBA.
Fig: D. The complete plantlet after removal of agar.

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