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RESEARCH ARTICLE

EFFECT OF DIFFERENT HORMONE COMBINATIONS ON CALLUS INDUCTION AND PLANT REGENERATION OF STRAWBERRY

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Abstract

An experiment was undertaken to examine the effects of different combinations of plant growth regulators for callus induction and plantlet regeneration in Strawberry (*Fragaria x ananassa* Duch.). Leaf discs derived from two months old strawberry plants were cultured on MS media supplemented with BAP. The cultures were incubated for 4 weeks in dark followed by another 4 weeks under 16/8 hr light regime. The effects of different concentrations of BAP (0, 1.5, 3.0 and 6.0 mg $L^{-1)}$ on callus induction were investigated. Among the concentrations 3.0 mg L^{-1} BAP showed the highest percentage (93.33%) of callus induction. To regenerate shoots, the calli derived from leaf discs were cultured on shoot induction media containing different combinations and concentrations of BAP (0, 1.5, 3.0 and 6.0 mg L^{-1}) and GA_3 (0.5, 1.0, 1.5 and 2.0 mg L^{-1}). The highest percentage of shoot regeneration (93.33%) and number of shoots (15.00) per leaf disc was found to be induced on the MS medium supplemented with 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3. The highest (83%) percentage of rooting in shoots was observed in MS medium in combination with 1.5 mg L^{-1} GA_3 and 1.0 mg L^{-1} IBA.

INTRODUCTION

The strawberry (*Fragaria x ananassa* Duch.) is an important commercial fruit of the Rosaceae family grown worldwide. Due to genetic heterozygosity, adaptability and plasticity of strawberry it becomes one of the most popular and valuable nutritious fruit of the world (Losina-Losinskaja, 1926; Staudt, 1999*a*). Fruits are richest source of bioactive phytochemicals, with high antioxidants (Wang *et a*,,. 1996; Heinonen *et al.*, 1998; Koşar *et al.*, 2004) and as a part of daily diet, it could be beneficial for human health (Hannum, 2004), therefore its cultivation and production is increasing year by year (Esitken *et al.*, 2010). According to nutrient database for standard reference the strawberry fruits are rich in vitamin C, B1, B2, protein, calcium, potassium, iron, and most of other nutritients essential for human health (Chieng-Ying *et al.,* 2009; Kafkas *et al.,* 2007). Because of food value and other importance of strawberry, the production and consumption rate in Bangladesh is increasing day by day. There are about 20 recognized species of strawberries in five chromosome groups (x = 7): ten diploids, four tetraploids, one pentaploid, one hexaploid and four octoploids (Staudt 1999; Jiajun *et al.*, 2005). The cultivated strawberry is an octoploid (2n = 8x = 56) produced by natural hybridization of *Fragaria chiloensis* L. P. Mill. and *Fragaria virginiana* Duch (Staudt and Dickore, 2001). Plant tissue culture is the science or art of growing plant cells, tissues or organs on artificial media by isolating them from the mother plant. It is based on the cell doctrine that states a cell is capable of autonomy and is potentially totipotent. In 1902, the the concept of *in vitro* cell culture was developed by German botanist Gottlieb Haberlandt (Krikorian *et al*., 1969). Regeneration protocols of strawberry are species specific to their regeneration capacity (Passey *et al.*, 2003), and different mixtures of growth regulators have been used for the regeneration of shoot from various explants (Schaart *et al*., 2002; Passey *et al*., 2003; Zhao *et al*., 2004 ; Yonghua *et al*., 2005; Biswas *et al*., 2009). Selection of the proper hormone combination, explants, and cultivar are the keys to successful regeneration of strawberry (Barcelo, 1998; Jimenez-Bermudez, 2002). Leaf tissue of strawberry has been studied and shown to have the greatest regeneration capacity (Jelenkovic, 1991; Nehra *et al*., 1990; Passey *et al*., 2003 and Popescu *et al.,* 1997). Callus production is also more prolific from the leaf tissue. Calluses induced from leaf disc explants of *in vitro* grown plants exhibited higher regeneration compared to those induced from greenhouse-grown plants (Khan and Spoor, 2004). Different hormonal combinations and leaf disc explants sources influence the number of regenerated plants (Adak *et al*., 2001). A pretreatment in darkness is vital for callus induction and plantlet regeneration (Popescu *et al.,* 1997). Therefore, regeneration of strawberry is influenced by explants, hormonal combinations, light and season of the crop grown. Plant cell culture has become an excellent method for plant cell differentiation as well as a supplementary technique for plant breeding programs through the uses of new and expanded genetic variability (Nakamura and Maeda, 1989). To get disease free healthy plant materials it is very urgent to develop a protocol for *in vitro* propagation of strawberry. In spite of plenty of information on tissue culture studies elsewhere in the world, reports of studies in Bangladesh are still not sufficient. That's why, the present study was carried out to develop an efficient regenerative protocol from leaf discs of strawberry in the shortest possible period with the optimum concentrations of growth regulators for selecting desirable plantlets for commercial cultivation.

MATERIALS AND METHODS

Plant materials and explant collection:

For the establishment of culture, the planting materials or explants (leaf) of *Fragaria x ananassa* Duch. were collected from Bangladesh Agricultural Research Institute (BARI), Rangpur.

Sterilization:

Explants were surface sterilized by using mercuric chloride ($HgCl_2$) as surface sterilizing agent, tween-80 and savlon inside the Laminar Air Flow chamber. To ensure aseptic condition under *in vitro*, all instruments, glasswares and culture media were sterilized by autoclaving with 15 Ibs/sq. inch (1.16 kg/cm²) pressure at 121º C temperature for 30 minutes.

Placement of explants on culture media:

Sterilized leaves were cut into strips (0.5 × 0.5 mm) avoiding the midrib and placed axial side down onto the semisolid MS (Murashige and Skoog, 1962) media with concentration of growth regulator BAP (0.0, 1.5, 3.0 and 6.0 mg L^{-1}). The pH of all media were adjusted to 5.7 before addition of agar and sterilized by autoclaving for 20 minutes at temperature 121°C and pressure 15lb. The culture vials containing explants were placed under dark condition in a room with controlled temperature (25±2º C) for the first four weeks followed by four weeks of 16hr light/day by white florescent tubes.

Regeneration of shoot and root:

After callus formation, each microshoots of 7-8cm long were aseptically transferred to MS medium supplemented with different concentrations of BAP and GA_3 for shoot initiation. Root formation was observed from the regenerated shoot in MS medium supplemented with different concentrations of BAP and IBA.

Data analysis: The collected data were analyzed by using the statistical program MSTST-C for analysis of variance (ANOVA) and mean separation.

RESULTS AND DISCUSSION

The leaf explants were used for induction of callus development in all of the culture media formulations. The cultured explants showed significant variation on days required for callus induction as well as percentage of callus induction by the influence of different concentrations of BAP. The least number of days required for callus induction was 45.67 at 3.0 mg L^{-1} BAP and maximum numbers of days (49.33) was required for 6.0 mg L^{-1} BAP. On the other hand, the highest percentage (93.33%) of callus induction was observed in 3.0 mg L^{-1} BAP and lowest percentage (80.67%) was found with 6.0 mg L^{-1} BAP. MS medium without BAP had no callus formation (Table 1) confirms the findings of Adel and Sawy, 2007; Biswas *et al.,* 2007; Sakila, *et al.,* 2007; and Harker *et al.,* 2000 who described that BAP is vital for the regeneration of strawberry.

However, the growth regulator concentrations significantly affected the shoot initiation**.** Table 2 shows that a combination of 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 performed better and needed least number of days (8.67) for shooting. Maximum number of days (18.0) was required in the combination of 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} GA_3. Secondly, the highest shoot initiation (93.33%) was observed with the supplementation with 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 to the medium. Again, the combination of 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 showed the highest number of shoots (15.00) per explant and the lowest number of shoots (3.33) per explant was observed in the combined concentration of 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 which is statistically similar to the combined application of 1.0 mg L^{-1} BAP and 1.5 mg L^{-1} GA_3 (Table 2). Similar shoot regeneration frequency was also reported by Mohamed *et al.,* (2007).

Furthermore, The combined concentration of 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 resulted in the longest shoot (2.0 cm) and the shortest shoot (0.60 cm) was observed of 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3 (Table 2).

The rooting of regenerated shoots was significantly affected by different concentrations growth regulators (Table 3). The rooting of shoots was non-significant in terms of days required for root initiation but percentage of shoots showing roots showed significant variation. The combination of 1.5 mg L^{-1} GA_3 and 1.0 mg L^{-1} IBA performed best and required least number of days (52.00) for rooting. Maximum number of days (59.00) was required for the combination of 1.0 mg L^{-1} GA_3 and 0.5 mg L^{-1} IBA. Similar rooting frequency was also reported by Mereti *et al.,* (2003). On the other hand, the highest root induction (83.00%) was observed with the combination of 1.5 mg L^{-1} GA_3 and 1.0 mg L^{-1} IBA and the lowest root induction (53.33%) was observed in the addition of 0.5 mg L^{-1} GA_3 and 0.5 mg L^{-1} IBA to the medium.

The tissue culture technology provides an alternative method of plant regeneration of economically important plants. At present, there is a great demand of strawberry in our country. Application of this technology can be an effective way of regeneration of strawberry plants.

Table1. Effect different concentrations of BAP on callus induction from leaf explants

Treatment (mg L^{-1}) BAP	Days required for callus induction	Percentage of callus induction
0.0	-	0.0d
1.5	46.33b	88.33b
3.0	45.67b	93.33a
6.0	49.33a	80.67c
CV (%)	1.56	3.57
LSD($_{0.05}$)	1.11	4.67

Means having common letter(s) are statistically identical at 5% level.

Table2. Effect of different concentrations hormone on shoot initiation

Hormone concentrations (mg L^{-1})		**Days required for shoot initiation**	**Percentage of explant induced shoot**	**Number of shoots explant^{-1}**	**Length of shoot (cm)**
BAP	**GA_3**				
1.0	0.5	12.33b-e	41.67g	3.33i	0.60b
	1.0	16.00a-c	56.00fg	4.67hi	1.30b
	1.5	16.67ab	50.00ef	3.33i	0.83b
	2.0	18.00a	61.60d-f	4.67hi	0.63b

1.5	0.5	13.33a-e	78.33a-d	9.33de	1.50b
	1.0	17.00ab	73.33b-f	12.00bc	1.43b
	1.5	10.67c-e	88.33ab	10.33cd	0.97b
	2.0	14.33a-d	63.33c-f	7.33fg	1.17b
3.0	0.5	8.67e	93.33a	15.00a	2.00a
	1.0	9.33de	86.67ab	13.67ab	1.56b
	1.5	12.67a-e	78.33a-d	9.00d-f	1.13b
	2.0	11.67b-e	73.33b-f	8.33ef	0.87b
6.0	0.5	15.00a-c	80.00a-c	10.00de	1.47b
	1.0	9.33de	88.33ab	9.33de	1.43b
	1.5	12.33b-e	76.67a-e	6.33gh	0.83b
	2.0	14.00a-e	73.33b-f	7.33fg	1.43b
CV (%)		21.02	12.89	14.13	23.45
$LSD_{(0.05)}$		4.63	15.75	1.70	1.37

Means having common letter(s) are statistically identical at 5% level

Table3. Effect of different concentrations of hormone on root initiation

Hormone concentrations (mg L^{-1})		Days required for root initiation	Percentage of shoots showing roots
GA_3	IBA		
0.5	0.5	58.33ab	55.33g
	1.0	55.00d	72.33c
	1.5	57.67a-c	69.00d
1.0	0.5	59.00a	63.33e
	1.0	55.33cd	80.00b
	1.5	56.00b-d	65.00e
1.5	0.5	57.00a-d	59.00f
	1.0	52.00e	83.00a
	1.5	55.00d	70.67cd
2.0	0.5	57.67a-c	64.33e
	1.0	55.33cd	79.33b
	1.5	54.67d	73.00c
CV (%)		2.43	2.25
$LSD_{(0.05)}$		2.307	2.64

Means having common letter(s) are statistically identical at 5% level

Plate 1.Callus from the leaf explant in MS medium supplemented with 3.0mg L^{-1}BAP after 45 days of incubation.

Plate 2.Callus from the leaf explant in MS medium supplemented with 1.5mg L^{-1} BAP after 45 days of incubation

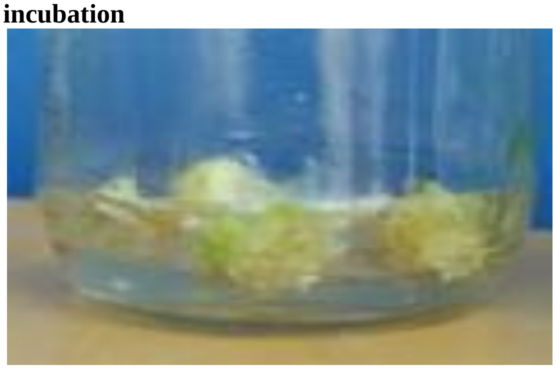

Plate 2.Callus from the leaf explant in MS medium supplemented with 6.0mg L^{-1} BAP after 45 days of incubation

Plate 4. Multiple shoots from leaf derived calli in MS medium supplemented with 3.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3

Plate 5. Multiple shoots from leaf derived calli in MS medium supplemented with 1.5 mg L^{-1} BAP and 1.0 mg L^{-1} GA_3

Plate 6. Multiple shoots from leaf derived calli in MS medium supplemented with 6.0 mg L^{-1} BAP and 0.5 mg L^{-1} GA_3

Plate 7. Root formation from the regenerated shoots in MS medium supplemented with the combination of 1.5 mg L^{-1} GA_3 and 1.0 mg L^{-1} IBA

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