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Induction of Callus Through Anther and Ovule Culture in Upland Cotton (*Gossypium hirsutum* L.)

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Abstract: Three cotton genoytypes Reshmi, Rehmani and TH-3/83 were studied for callus induction under two concentrations (3 mg/l and 4 mg/l) in four auxins viz. 2,4-D, IAA, IBA and Kinetin. Immature ovules and anthers of cotton varieties were utilized as explant material on Murashige and Skoog (MS) media The best callus induction was observed from the ovules rather than the anthers. A high percentage of regenerable callus was produced in Reshmi variety through immature ovules observed in the medium supplemented with 3mg/l. Rehmani followed Reshmi variety which produced 88% callus with 3mg/l, whereas callus induction through immature anthers, the callus formation was less from both concentrations (3 mg/l and 4 mg/l). However, when the amount of 2, 4-D was increased in the medium the rate of callus formation was reduced.

Key words: Missing

INTRODUCTION

Cotton is great economic importance for our country as it acts cerebral column in our agricultural and industrial development, employment generation and foreign exchange earnings through export of its raw materials as well as furnished products. Pakistan is the fourth major cotton producer and exporter in the world where it grows on about 12% of the total cultivated area. Besides its use in textile industry, cotton also provides food in the form of edible oil and cotton seed cake for animal feed. Its contribution in oil production amounts to 60-70% of the local production of the edible oils in the country [1].

Cotton is one of the most important fiber crops in the world. Genetic improvement of cotton through conventional breeding is limited by several factors such as lack of useful variation and long time periods that are required. Although plant biotechnology is an attractive means for improving cotton, its use requires an effective regeneration system from somatic tissues of cotton plants cotton. Plant biotechnology describes a precise process in which scientific techniques are used to develop useful and beneficial plants Establishment of an efficient tissue culture in vitro protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. Important trait of plants. Regeneration of plants from callus is an indispensable step for breeding when the strategies such as anther and ovule culture are used. Also, production of callus and its subsequent regeneration is the prime steps of crop plant to be manipulated by biotechnological means [2]. Nevertheless, it has been reported that there are large variations among varieties of the same sub species in terms of in-vitro culture response. Davidonis and Hamilton [3] first described plant regeneration from two-year old callus of Gossypium hirsutum L. cv Coker 310 via somatic embryogenesis. Since then, significant progress has been reported in cotton tissue culture [4,5]. Anther and ovule culture have advantage of production of haploid plants, production of homozygous diploid lines through chromosome doubling, thus reducing the time required to produce inbred lines.

The aim of this study was to excise anthers and ovules from three local cotton varieties viz. Reshmi, Rehmani and TH-3/83 which were cultured on M.S. medium with different concentration of 2,4-D (3 mg/l and 4 mg/l), supplemented with IAA, IBA and KIN for induction of callus.

MATERIALS AND METHODS

The seeds of three commercial cotton varieties viz. Reshmi, Rehmani and TH-3/83 were received through Cotton Section of Agriculture Research Institute, Tando Jam. These varieties were grown in the tissue culture

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laboratory of Department of Biotechnology, Sindh Agriculture University, Tando Jam. After some days when the flowers reached the bud stage immature anthers and ovules were excised as explants material for callus induction. The anthers and ovules of each variety were removed from the flower buds after surface sterilization with sodium hypochloride under aseptic conditions. The anthers and ovules were excised from the bud under aseptic conditions and cultured in tubes containing M.S. medium with 3mg/l and 4mgl 2,4-D supplemented with different concentration of IAA, IBA and KIN for induction of callus. These cultures tubes were kept in the growth room at 280C for callus induction.

RESULTS AND DISCUSSION

Plant tissue culture has emerged as the rapidly developing science because of its proven success in agricultural improvements that is elimination of disease, enhance multiplication rates of selected clones of importance, evolution of new varieties in a reduced span of time and a shortened breeding cycle. There is an imperative need to transfer entire agricultural production into a science and technology based system.

As such, seed of three local varieties of cotton viz. Rehmani, Reshmi and TH-3/83 were grown at Tissue culture laboratory at the Department of Biotechnology, Sindh Agriculture University, Tando Jam. Immature ovules and anthers of cotton were used as explant material for callus induction. The explants were excised in culture tubes containing M.S. medium supplemented with different concentrations of 2,4-D + IAA, IBA and KIN 3mg/l and 4mg/l. In this way 300 bottles were cultured for each anther and ovule. The cultured tubes were incubated in growth room at the temperature of 280°C.

For callus formation the concentration of 2,4-D at 3mg/l gave good results as compared to 4mg/l. Increasing the amount of 2,4-D reduced the amount of

Table 1: Callus formation in culture tubes by using ovules as explant material on M.S. medium with IAA 1.0mg/l,IBA 0.1 mg/l and Kin 1.0 mg/l

Media composition		Cotton varieties		
M.S+2.4-D 4mg/l +IAA, IBA and Kin	Variety	Reshmi	Rehmani	TH-3/83
	No of ovules cultured	50	50	50
	Callus formation	44	40	37
	No. of contaminated culture	06	10	13
	%age of callus formed	88%	80%	74%

Table 2: Callus formation in culture tubes by using ovules as explant material on M.S. medium with IAA 1.0mg/l,IBA 0.1 mg/l and Kin 1.0 mg/l

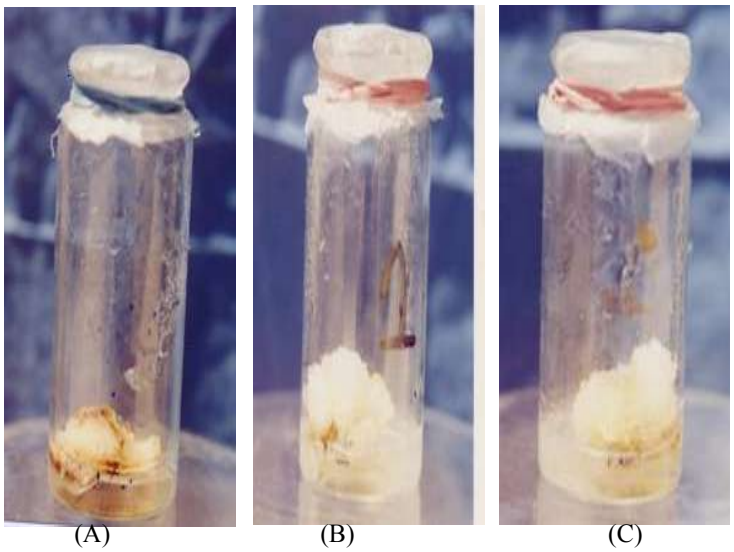
Media composition		Cotton varieties		
M.S+2.4-D 3mg/l +IAA, IBA and Kin	Variety	Reshmi	Rehmani	TH-3/83
	No of ovules cultured	50	50	50
	Callus formation	45	42	38
	No. of contaminated culture	05	08	12
	%age of callus formed	92%	88%	76%

Table 3: Callus formation in culture tubes by using anthers as explant material on M.S. medium with IAA 1.0mg/l,IBA 0.1 mg/l and Kin 1.0 mg/l

Media composition		Cotton varieties		
M.S+2.4-D 3mg/l +IAA, IBA and Kin	Variety	Reshmi	Rehmani	TH-3/83
	No of ovules cultured	50	50	50
	Callus formation	31	28	25
	No. of contaminated culture	19	22	25
	%age of callus formed	62%	56%	50%

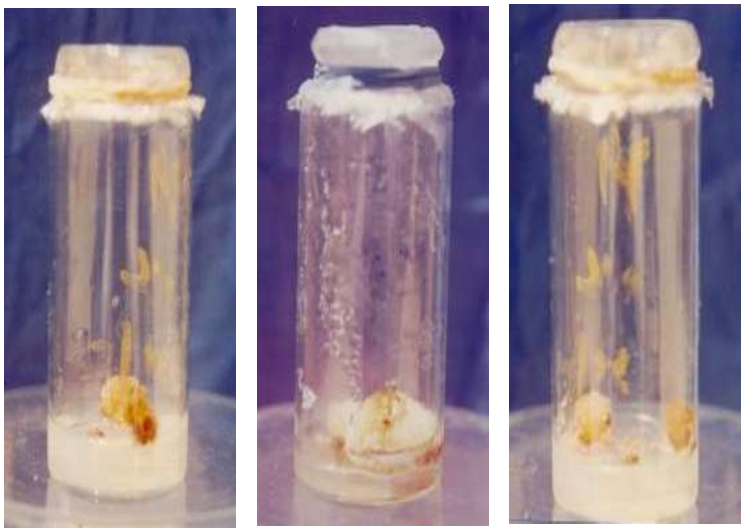
Table 4: Callus formation in culture tubes by using anthers as explant material on M.S. medium with IAA 1.0mg/l,IBA 0.1 mg/l and Kin 1.0 mg/l

Media compostion		Cotton varieties		
M.S+2.4-D 3mg/l +IAA, IBA and Kin	Variety	Reshmi	Rehmani	TH-3/83
	No of ovules cultured	50	50	50
	Callus formation	29	24	23
	No. of contaminated culture	21	26	27
	%age of callus formed	58%	48%	46%



(A) (B) (C)

(A) Callus formation through ovule culturein Reshmi variety
(B) Callus formation through ovule culturein Rehmani variety
(C) Callus formation through ovule culture In TH-3/83 variety



(A) (B) (C)

(A) Callus formation through anther culture from Reshmi variety
(B) Callus formation through anther culture from Rehmani variety
(C) Callus formation through anther culture from TH-3/83

callus formation. In Table 1 & 2 shows that the callus formed from ovules at the rate of 3mg/l was 92% for Reshmi variety. Whereas Rehmani produced 88% callus and TH-3/83 produced 80% callus. However, for 4mg/l 2,4-D 88% callus for Reshmi variety, 80% Rehamani and 74% callus by TH-3/83.Similar results were reported by Jayasahankar *et al.* [6].

The immature anthers also revealed better response to 3mg/l as compared to 4mg/l. The Table 3 and 4 shows that 62% callus formed from anther culture by the variety Reshmi, 56% by Rehmani and 50% by TH-3/83. Whereas 4mg/l concentration of 2,4-D showed lowest callus induction.

The highest rate of callus was obtained from ovules at 3mg/l 2, 4-D concentration which was of good quality and more in weight. Reshmi variety response was well in all the treatments as compared the two varieties. The future researchers can further work to induce regeneration of plantlets from the callus so that new genotypes could be obtained.

CONCLUSION

For callus induction both the ovule and anther explant in the media containing 3 mg/l 2, 4-D produced more efficient frequency of callus as compared to 4mg/l. The variety Reshmi produced better response for callus induction through ovule culture as compared to other to varieties in which highest %age was 92% with 3mg/l 2,4-D and 88% with 4mg/l 2,4-D .Rehmani also produced optimally maximum callus with ovule as explant (88% and 80%). Similarly, for the anthers as explant showed better response for callus for the variety Reshmi at 3 mg/l 2,4-D (62%). TH-83/3 performed minimum% age of callus as compared to other varieties with 3mg/l 2,4-D and with 4mg/l 2,4-D .Hence it is concluded that varieties with good response to callus can be used to induce regeneration of plantlets in reduced time and new genotypes could be.

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