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In Vitro regenerated disease resistant cell lines of Pennisetum americanium against Downy Mildew

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ABSTRACT

Cereal grains were collected from healthy and infected plants of cultivar BJ-104 infected by *Sclerospora graminicola.* Explants (2-3 mm) were cutred in MS medium containing additional chemical ingredients to raise callus culture. 2, 4- D (2-5 mg/1) + Kinetin (0.5 mg/1) produced embryogenic and non- embryognic calli,the former developed emryoids. On ¾ MS medium with Kinetin (0.4 mg/1), callus produced shoots from embryos. Addition of IAA (2.5 mg/1) in the medium without cytokinins produced roots from distal ends of shoot segments when transferred to fresh ½ MS medium. Embryoids from culture were regenerated in modified MS medium containing 150 m1/1 fungus and segments were rooted in the medium with 2.5 mg/1 IAA and 2.5 mg/1 ascorbic acid. Plantlets were prepared for hardening, acclimatization and establishment for autotrophic adaptation. Plantlets were transferred to the soil via vermiculite + sand soil medium. Regenerated plants (1 month) were tested for resistance to downy mildew (i) in oospore mixed soil and (ii) grains in ¼ MS medium with 150 m1/1 fungus filtrate. Surviving plantlets were raised as disease resistant

Keywords: *Pennisetum americanum*, *Scelrospora graminicola, Downy mildew*, *In vitro* regenerated disease resistant cell lines

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INTRODUCTION

Literature with in vitro regeneration of monocotyledons especially the cereals is relatively scanty which entails several reasons like lack of diffusion and psysiological gradients of substances which induce clustering of cells leading to embryogenesis (Ross and Thorpe, 1973) physiological state of the calli and carry out effect of auxin from inoculum to substrate that influences embryo differentiation(Walperin, 1970) and proper balance of nutrient media for initial proliferation of the tissues initiating embryogenesis (Rangan, 1976). Relatively less number of millets and cereals which have been brought into callus which regenerated into complete plantlets following application of plant hormone from high concentration to auxin free medium. Regenerated plantlets of panicum, Paspalum, pennisetum and Elusin, Lasiurus are transferred to soil and grown to maturity (Rangan, 1976; kakkar and Shekawat, 1989).

Carlson (1980) selected plant cells and protoplast to culture for raising resistant cells lines which were regenerated with altered response to toxicity of the pathogen. Considerable interest has been generated in the potentiality of callus culture for regeneration and development of disease resistant germplasm. The prospective studies also included plant cell, tissue, protoplast culture, and somatic hybridization technology involving new dimensions for genetic improvement including micro & clonal propagation of plants (Arya and Shekawat, 1986). The technique also provided an opportunity to study the inter- relationship of host and pathogen in establishment, growth and development of these plants (Rhode, 1983) in cutures containing filterates of *Scherospora graminicola* from the axemic culture (Tiwari and Arya, 1969).

MATERIALS AND METHODS

The grains were collected from healthy, partially proliferated spikelets and excessively proliferated infected tillered shoots of cultivar BJ-104 infected with *Scelrospora graminicola.* The suppressed ear-head formation was considered as the most advanced stage, of downy mildew infection. Explants of 2-3 mm size were cut from suppressed anh healthy ear-heads. Explants were surface sterilized with 0.2% ethyl mercuric chloride and rinsed with sterile distilled water containing antioxidant solution 2-3 times (ascorbic acid, 100 mg/1, Citric acid, 50 mg/1;) and PVP (Polyvenyl pyrrolidon, 10 mg/1). The explants were transferred to the MS medium (Murashige and Skoog, 1962) with additional chemical ingredents (2, 4-D), Kinetin, ascorbic acid and coconut water) to raise the callus culture (Vasil and Vasil, 1982).

RESULTS

Callus Initiation

Callus initiation was obtained after six days of inoculation of grains but explants from infected organs induced callus after 14 days of inoculation.

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Different concentrations of auxins 2, 4-D and NAA used were: 2.0 to 10.0 mg/1 and 0.1 to 6.0 mg/1, respectively and cytokinins, kinetin and BAP, 0.05 to 2.0 mg/1. In another experimenta 2, 4-D (1.5 to 3.5 mg/1) + kinetin (0.05 to 1.0 mg/1 were incorporated in MS medium. Inoculated cultures were kept in the culture chamber at 26+2°C at 2000 lux light mixing flurescent and incandescent light in the ratio of 4:1.

Results of callus initiation with different combinations of auxins and cytokinins showed that 2, 4-D (2.5 mg/1) combined with kinetin (0.5 mg/1) was most suitable for callus production (980 mg). Two types of calli were distinguished viz. Embryogenic with slow growth and non – embryogenic with relatively fast rate of growth. Slow growing callus at the advanced stage developed embryoids after 6 days of growth which turned light green in continous light (Fig 1).

Callus Morphogenesis

Embryoids from healthy and diseased calli were transferred seperately to ¾ strength MS medium without 2, 4-D supplemented with ascorbic acid(25 mg/1) and coconut water(150 m1/1). Concentration of kinetin and BAP used seperately and combinedly were 0.1 to 1.5 mg/1. 10 replicates were used for each treatment and tissues were grown for 3 successive passages at the same concentrations to remove the residual effect of pre-treatment and tissues were grown for 3 successive passages at the same concentrations to remove the residual effect of pre-treatment of culture and they were subcultured every 2 weeks on fresh medium. The cultures were kept in the light culture chamber at 28±2°C and 3000 lux light mixing flurescent and incandescent in the above ratio.

On transfer of callus to ¾ MS medium supplemented with kinetin and BAP, seperately in different concentrations produced shoots (Table 1). Kinetin with 0.4 mg/1 showed best results and 40-70 percent embryos produced shoots (Fig 2). When kinetin and BAP were combined shoot production results were poor (10-18 percent).

Table-1: Effect of cytokinins on callus morphogenesis of pearl millet.

Concentration used (mg/1)	CYTOKININ			
	Kinetin		BAP	
	Nature of Response	% of response	Nature of response	% of response
0.1	S +	2	NR	-
0.2	S + +	16	S +	8
0.3	S + + +	28	S +	12
0.4	S + + + +	72	S + +	22
0.5	S + + + +	42	S +	11
0.6	S + + +	31	S + + +	29
0.7	S + +	21	S + + + +	42
0.9	NR	-	S +	7
0.9	NR	-	NR	-
1.0	NR	-	NR	-

NR = No response ; S = Shoot
\+ = Poor shooting ; + + = moderate shooting
+++ = Good shoting ; + ++ = Excellent shooting

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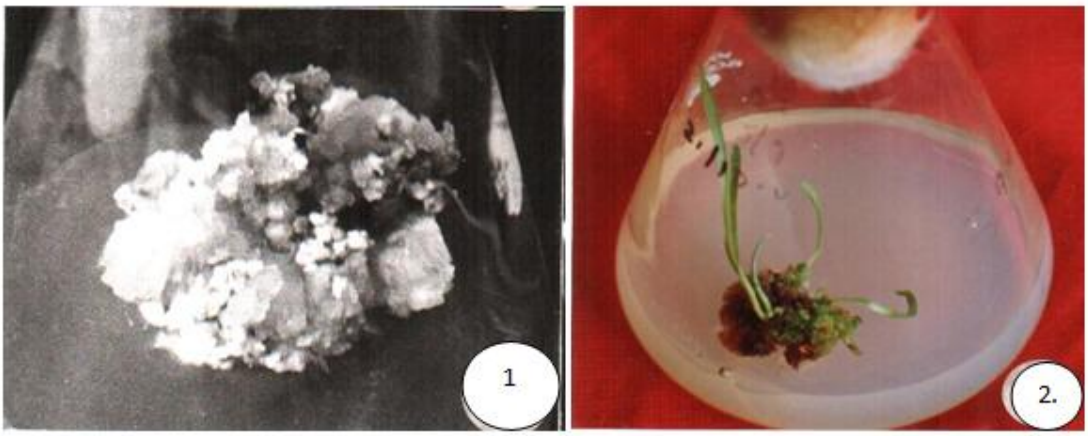

Fig.1: Photograph showing embryogenic callus raised from grains on MS medium supplemented with 2,4-D (2.5 mg/L), kinetin (0.5 mg/L), ascorbic acid (25 mg/L) and coconut water (150 ml/L).

Fig.2: Photograph showing shoot formation in callus morphogenesis on auxin free MS medium with kinetin (0.5 mg/L), ascorbic acid (25 mg/L) and coconut water (150 ml/L).

Fig.3: (a-b) Photograph showing root initiation on MS medium with IAA (2.5 mg/L). Complete plantlets with roots and shoots.

Root Induction:

Cytokinins showed no response to root induction. Only auxins were used, therefore for root initiation. On increase in the concentration of IAA upto 3.0 mg/1, rhizogenesis took place directly from the embryoids or from the distal ends of the shoot segments thrust into the

medium. The optimal was 2.5 mg/1 (Fig 3 and Table 2). IBA (2.0 mg/1) also gave good rooting of the shoot segments.

Table-2: Effect of auxins on root induction in shoot segments of pearl millet.

Concentration used (mg/1)	AUXINS			
	I AA		I BA	
	Nature of Response	% of response	Nature of response	% of response
0.5	NR	-	NR	-
1.0	R +	9	R +	7
1.5	R +	8	R + +	18
2.0	R + + +	38	R + + +	25
2.5	R + + + +	56	R + +	16
3.0	R + +	28	NR	-
3.5	NR	-	NR	-
4.0	NR	-	NR	-
5.0	NR	-	NR	-

NR = No response R = Shoot
\+ = Poor shooting ++ = Moderate shooting
+ + + = Good shoting + + + + = Excellent shooting

IN VETRO HARDENING AND ACCLIMATIZATION OF TISSUE CULTURE REGENERATED PLANTS

In vitro regenerated plantlets were ready for transfer from aseptic culture to the soil. These were prepared for further growth, hardening, acclimatization and establishment for autotrophic adaptation before transfer to the field.

Rooted plants were removed after 2 weeks of culture from the rooting medium and transferred to the reduced salt concentration (1/4) MS medium eliminating vitamins, carbon source i.e sucrose and growth regulators. The tubes with plantlets were kept in the glass house under bell jar covered with wet cloth to keep the atmosphere humid with 80-90 percent relative humidity. The temperature in the glass house varied from 25 to 40°C and light intensity upto 4000 lux. The lower half of the tube was covered with black carbon paper to facilitate root development and growth.

After 10 days, the plantlets were carefully dissected out from the medium without causing any damage to delicate root system. They were then washed thoroughly under running tap water to cleanse the plantlets. The plantlets were treated with Bavistin (0.05 percent) for 10-15 min. and transplanted to 6" earthen pots containing vermiculite and sandy soil in the ratio of 3:2. The pH of the soil was 4.8 to 6.0. The pots were watered with tap water once or twice with Hoagland solution. As usual the plantlets were covered with beakers with wet filter paper pads for first 15 days.

Success in regenerating plants was 55 percent (Fig.4) The results suggested that this method might be developed for *in vitro* propagation of *P.americanum* which might be used for genetic manipulation of the species.

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Fig.4: Photograph showing production of disease free (Resistant) plants transferred finally to soil.

DEVELOPMENT OF RESISTANT CELL LINES

The technique used for regeneration has been used for development of disease resistant lines and the results obtained are presented in the following:

(A) Embryoids formed in embryogenic dual culture were isolated and grown on modified MS medium with different concentrations of $MnCl_2$ (50-400 mg/1) + Kinetin (0.5 mg/1) + ascorbic acid (25 mg/1) + coconut water (150 ml/1) + Bavistin (0.05%) for morphogenesis of embryoids. Cultures were kept in the culture chamber at 28 ±2°C and 3000 lux light.

The embryoids turned green, regenerated and formed shoots in continuous light comprising incandescent and fluorescent light in the ratio of 1:4. The results showed that $MnCl_2$ (200-250 mg/1+ + Bavistin (0.05%) were found best for controlling growth of the fungus.

The growing shoot segments (3-5 cms) were transferred to fresh ½ strength MS medium for root induction with 1AA (2.5 mg/1) + ascorbic acid (25 mg/1) + $MnCl_2$ (250 mg/1).

The rooting was initiated to form plantlets after incubation for 6-7 days at 28±2°C (Fig 4). Plantlets with developed root system were transplanted to 6" earthen pots after following the processes of root hardening, acclimatization and transplantation which have already been discussed.

(B) Embryoids formed in embryogenic normal culture were picked up and transferred to MS medium containing kinetin (0.5 mg/1) + ascorbic acid (25 mg/1) + coconut water(150

ml/1) + filtrate of the axenic culture of the fungus (150 ml/1). The cultures as usual were kept in light culture chamber. The embryoid, were regenerated to form shoots in continuous light. Regenerated shoots were transferred to rooting medium with 1AA (2.5 mg/1) + ascorbic acid (25 mg/1). The roots were formed after incubation for 7-10 days at 28±2°C and after usual processing were transferred to the soil via vermiculite + sand soil medium.

The regenerated plants (1 month old) were observed for their resistance to downy mildew. Two experiments were set for this purpose.

(a) Plantlets (2 week old) growing in the vermiculite + sand soil were transplanted to 6"earthen pots in oospore mixed soil. The pots were kept in the glass-house covered with polyethylene cloth to maintain 100% humidity for first 72 hrs. and then watering for the first one week with distilled water then with tap water. Grains were collected from healthy plants which flowered in 2 months and sown for further testing.

(b) Grains collected from above experiment were taken and sown and regenerated *in vitro* condition. The plantlets were transferred to the modified ¼ MS medium with 150 mg/1 filtrate of the fungus and surviving plantlets (50%) were allowed to grow for 2 weeks. Afterward plantlets were taken out washed with distilled water and transferred to soil for growth and flowering which took place in 2 months. Grains formed were collected.

It was observed that 90-95% plants upto F_2 generation showed their resistance to downy mildow disease and grains collected formed the disease free germ plasm.

CONCLUSION

It may be concluded from the results presented in the paper that one of the important methods for controlling the downy mildew disease of pearl millet in countries like India is to raise the disease free and resistant germ plasm through tissue culture techniques. The crop can be safeguarded from the disease by use of $MnCl_2$ (200 mg/1) spray and seed dressing with 0.05% Bavistin before putting it in the soil. This improved agricultural practice will so a long way to safeguard the purity of the seed in so far its disease resistant character is concerned.

REFERENCES

[1] Arya, HC and Shekawat NS. Forest Ecol Management 1986; 16: 201-208.
[2] Carlson PS. Blue roses and black tulips: is the new plant genetics only ornamental? In: R.C. Staples and R.J.Kular (ed.), linking research to crop production, Plenum, New York 1980; 63-77.
[3] Halperin W. Embryos from somatic plant cells, In: A.Padykula (EdS.), control mechanism, in the expression of cellular phenotypes, Academic Press, Newyork 1970; 169-191.
[4] Kakkar A and Shekawat NS. Ann Bot 1989; 64.
[5] Murashige T and F Skoog. Physiol Plant 1962; 15: 473-497.

[6] Rangan TS. Pflanzen – Physiol 1976; 78: 208-216.
[7] Rhode LH. Mycorrhizae, In: D.A. Evans et al. (ed.) Hand book of plant cell culture. McMillan publishing Co., New York 1983; 1: 924-940.
[8] Rose NK and TA Thorpe. Plant Cell Physiol 1973; 14: 473-480.
[9] Vasi V and Vasil IK. Amer J Bot 1982; 69: 1441-1449.