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Tissue Culture response of Local Varieties of Rice (*Oryza sativa* L.) of NWFP

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Abstract: Four rice genotypes i.e. Swat I, Swat II, Dilrosh 97 and Pakhal were tested for their callus induction frequency and their subsequent regeneration from different explants on a variety of media combinations. Swat I appeared to be the most responsive genotype to callus induction followed by Swat II, Dilrosh 97 and Pakhal respectively. Swat I and Swat II produced high amount of callus compared to other genotypes. Callus induction frequencies ranged from 68.88 to 57.70 percent in Swat I, 60.00 percent in Swat II, 52.20 percent in Dilrosh 97 and 40.00 to 42.20 percent in Pakhal from scutella on two different media. Similar response was also found for root induced calli. However, N6 medium containing 2 mg/l of 2,4-D was found to be optimum for callus induction irrespective of the genotypes and explant studied. All varieties showed significant differences in regeneration from two and three week old calli. RM medium containing MS salts and vitamins, 2 gm casamino acid, 1 mg/l NAA, 2 mg/l BAP and 30 gm sorbitol gave comparatively higher regeneration response than the other two media tested. Plants regenerated were grown in hormone free MS medium for vigorous rooting and subsequent transfer to soil.

Key words: Rice, callus, regeneration, explant, hormones

Introduction

Rice is one of the most important food crops of the developing world and rice genetic improvement through breeding has proven to be an effective mechanism for delivering the benefits of science and technology to hundreds of millions of poor people. Considerable progress has been made in the development of cellular biology and molecular genetic techniques that can be applied to the genetic improvement of rice. Tissue culture techniques such as anther culture, embryo rescue and use of somaclonal variants have contributed to the release of new rice varieties.

In vitro tissue culture is a prerequisite for plant transformation and the prerequisite of *in vitro* culture is the identification of tissues or cells, which are competent for regenerating whole plant, and regeneration of plant is possible via somatic embryogenesis or organogenesis.

The present study was carried out on four varieties widely grown in irrigated areas of NWFP. Although tissue culture studies for rice improvement in Pakistan has been reported earlier by Zafar *et al.* (1992) and Khanum *et al.* (1997), but no study was conducted on the varieties grown in NWFP. Different explants were used for the induction of regenerable rice culture including mature seed, scutellum and root induced callus. The present study showed genotypic variation in callus formation and regeneration.

Materials and Methods

Varieties of Rice (*Oryza sativa*): Seeds of *Oryza sativa* L varieties SwatI, SwatII and Dilrosh 97 were obtained from Agriculture Research Institute, Mingora, Swat and Pakhal from Dhodial Rice Research Station.

Preparation of Explant

Dehiscing and sterilization of Mature Seeds: Seeds of *Oryza sativa* were carefully dehiscid, washed with 70% Ethanol for two minutes then sterilized with 50% Bleach (Sod,hypochloride) for 10 minutes with shaking and washed 5 times with autoclaved distilled water and kept on callus induction medium.

Preparation and Sterilization of Tissue Culture Media: The media used in tissue culture studies were modified according to the compositions described by Ghareyazie *et al.* (1997). Callus induction and regeneration media were primarily based on the modification of formulation developed by Murashige and Skoog (1962) and Chu *et al.* (1975). Two media, LS (supplemented with 2.5 mg/l 2,4-D) and N6 modified (supplemented with 2 mg/l 2,4-D) were selected for callus induction. These media were solidified with 0.25-0.4% phytigel (Sigma). The pH was adjusted to 5.6-5.8 with one Normal HCl or one Normal NaOH prior to sterilization. The pH was always adjusted before the addition of phytigel and after the addition of agar. All media were sterilized at 121 °C at 15 lbs. psi for 15 minutes in an autoclave. All tissue culture work was carried out in a sterilized environment in a laminar airflow cabinet (Backers Company Limited).

Callus Induction and Regeneration from Different Explants:

Embryogenic calli were induced from scutellum of the seed cultured for 2-3 weeks on N6 modified and LS medium and isolated swollen scutella from growing seeds were used for regeneration studies. Callus was induced from roots. Seeds were cultured on N6 modified medium and kept in the dark for 3-5 days, A 1-cm length piece of the initiated and elongated seminal and some nodal roots were taken for incubation on N6 modified and LS medium. After 2-3 weeks the callus induced from roots was used for regeneration studies. Callus induced from scutella and roots were then transferred to MS based regeneration media supplemented with different concentrations of phytohormones. Three media RM (MS salts + MS vitamins + 2 gm/l casamino acid + 1 mg/l NAA and 2 mg/l BAP + 30 gm/l sorbitol), RK (MS salts + 2 mg/l BAP and 0.1 mg/l Kinetin) and SP (MS salts + 0.3 mg/l BAP + 5 mM spermidine) were used for regeneration purpose. The calli were kept in light 14h days/10h night cycle at 25 ± 2 °C. Regenerated plants were transferred to MS medium for production of vigorous rooting in magenta vessels (Sigma).

Results and Discussion

Callus Initiation: For successful regeneration of different rice genotypes, the important parameters have been found to be source of explant, genotype and medium.

Callus Induction Response on Different Media from Different Tissue Explants:

Two explants, scutellum and roots of four varieties of rice were tested for callus initiation on two media. Scutellum calli of the Swatl, SwatII and Dilrosh 97 were not much defined and were hairy similar to results of Abe and Sasahara (1982). On LS medium the scutellum callus induction of SwatII (60%±1) was high followed by Swatl (57.7%±1.4), Dilrosh 97 (52.2%±0.66) and pakhal (42.2±1.46). Whereas, these varieties gave better callus induction response on N6 based medium than LS medium. Table 1 shows that Swatl variety gave the highest callus induction response (68.88±1.33) than other varieties, SwatII variety (60±2.08) Dilrosh 97 (52.2±0.66) and Pakhal (40±1.5). Callus induction response of primary roots was tested on the same media and on both media root had similar and lower response to callus induction as clear from the Table 1.

Regeneration of Rice: Four varieties tested for callus induction were also tested for regeneration. These varieties were analyzed for regeneration ability on three different media. It was found that Regeneration of rice depended on the type of callus (source of callus), age and variety etc. (Fig. 1).

Regeneration of Rice Plants from Scutellum Derived Calli:

Regeneration studies were conducted on 2 week and 3 week old calli. It is clear from Table 3 that three weeks old calli gave better regeneration than 2 week old. Swatl variety gave 66.66%±1.4 regeneration when 3 week-old calli were used. Three week old calli of SwatII gave 46.66%±1.64 regeneration. Two week old calli gave moderate regeneration response (20%-40%). Over all scutellum derived calli gave better regeneration response on RM than the two RK and SP media. However, Swatl and SwatII calli of 2 and 3 week old gave the least regeneration response 10-12% on SP medium. While Dilrosh and Pakhal gave good regeneration response on SP medium (33.33-35%), which showed that different varieties responded differently to different media (Fig. 2).

Table 1: Callus formation of two explants, scutellum and root, of four different rice varieties on two different media

Variety	Callus induction on LS medium + 2.5 mg/l 2.4-D(%)		Callus induction on N6 medium + 2 mg/l 2.4-D (%)	
	Scutellum	Root	Scutellum	Root
Swatl	57.70±1.40	43.33±1.15	68.88±1.33	47.77±1.20
SwatII	60.00±1.00	48.88±0.77	60.00±2.08	42.22±1.24
Dilrosh97	52.20±0.66	37.77±0.66	52.20±0.66	41.11±0.66
Pakhal	42.20±1.46	30.00±1.00	40.00±1.50	33.33±0.57

Table 2: Regeneration of 2 week old calli of different varieties of rice (*Oryza sativa* L.)

Varieties	RM		RK		SP	
	Scutellum	Root	Scutellum	Root	Scutellum	Root
	Swatl	50.00±0.66	46.66±0.66	23.33±0.76	23.33±0.76	10.00±0.66
SwatII	33.33±0.76	36.66±1.0	20.00±0.67	33.33±1.20	10.00±0.14	6.60±0.66
Dilrosh	33.30±1.6	33.3±0.50	23.33±1.02	26.66±0.76	23.33±1.06	23.33±0.76
Pakhal	10.00±0.67	20.00±0.50	3.3±0.33	26.66±0.56	35.0±1.76	20.00±0.66

Table 3: Regeneration of 3 week old calli of different varieties of *Oryza sativa*

Varieties	RM		PK		SP	
	Scutellum	Root	Scutellum	Root	Scutellum	Root
	Swatl	66.66±1.40	46.66±1.20	26.66±0.76	23.33±0.14	13.33±0.76
SwatII	46.66±1.64	36.66±1.66	26.66±0.66	26.66±1.66	10.00±1.43	6.60±1.40
Dilrosh	46.66±0.66	36.66±1.66	33.33±.76	26.66±0.76	33.33±1.50	26.6±1.52
Pakhal	15.00±0.76	25.00±0.60	6.60±0.66	26.66±1.66	35.00±0.56	30.00±0.55

Regeneration of Rice from Root Tissue Induced Calli: Root tissue induced calli were also used for regeneration purpose. Two and three week old calli induced from root were investigated on the same three media. It was observed that root induced calli regeneration response was lower than scutellum derived calli (6-46%) as shown in the Table 2 and 3. Swatl variety gave the highest regeneration response (46.66%±0.66) on RM medium followed by SwatII variety (36.66%±1.66). On RK medium the regeneration response of root tissue induced calli was moderate (6%-33.33%). On SP medium Dilrosh 97 and Pakhal gave better regeneration than Swatl and SwatII (24%±1.3 and 25±0.76 respectively).

On the whole, all four varieties gave good response on RM medium (25-46.66%) and poor performance on SP medium (7-30%). Whereas, among varieties, Swatl gave better regeneration on all three media (13%-70%) and Pakhal gave poor regeneration (6%-30%).

Tissue Culture of Local Varieties of Pakistan: Four local varieties Swatl, SwatII, Dilrosh, 97 and Pakhal were studied for their *in vitro* tissue culture response, as the three varieties Swatl, SwatII and Dilrosh 97 are commonly grown varieties of N.W.F.P., which were studied because these information may be needed in context of crop improvement and genetic

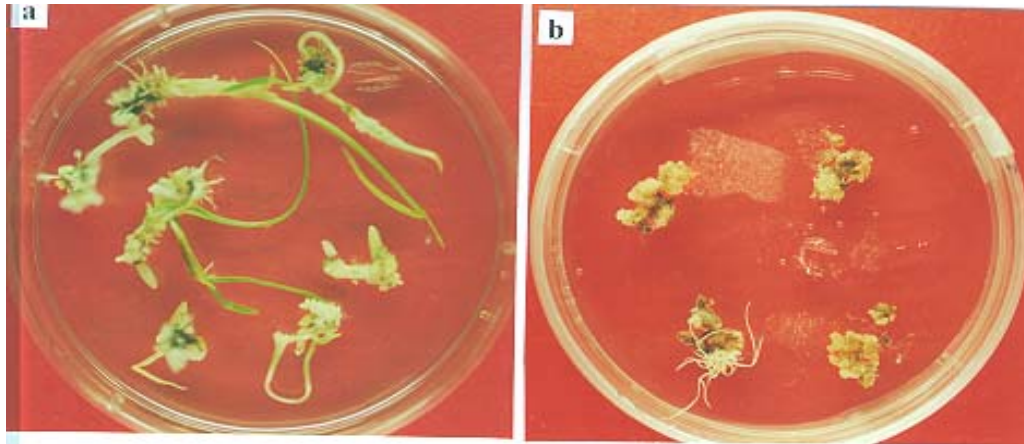


Fig. 1(a-b): Regeneration of Local Rice Varieties
(a) Shoot and formation on N6 modified media, (b) Rooting of callus on N6 modified media

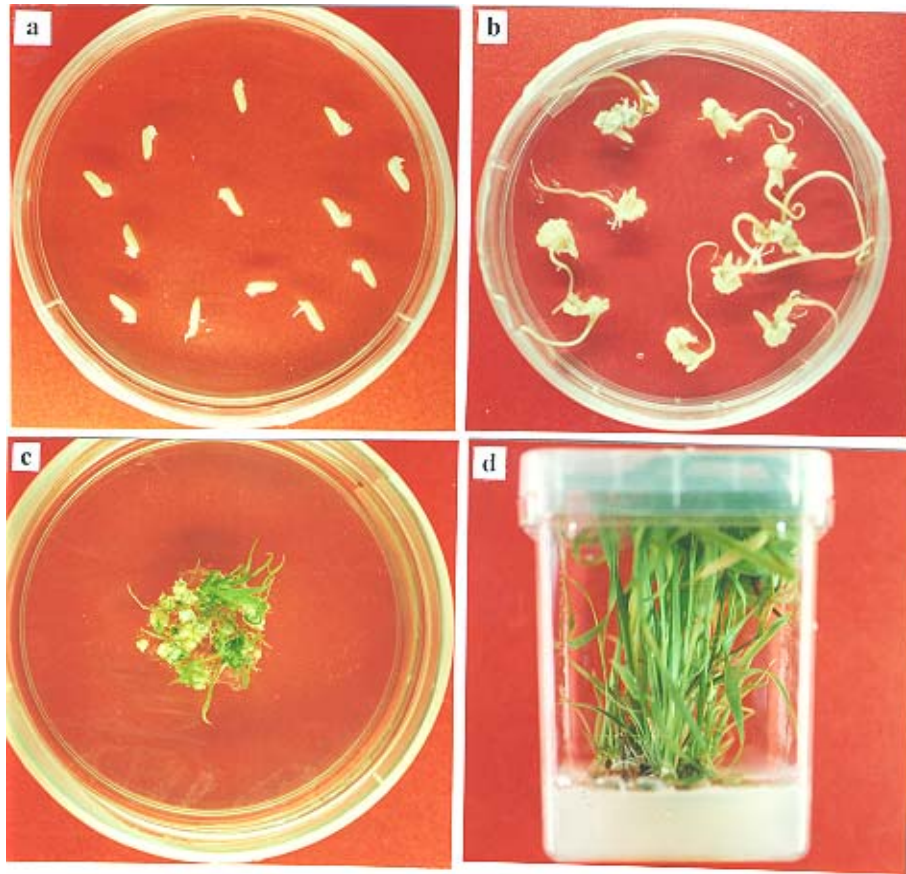


Fig. 2(a-b): Different stages of Regeneration of local Rice varieties from scutellum derived calli
(a) Incubation of seeds on callus inducing N6 medium, (b) Embryogenic callus formation after 2 weeks
(c) Regenerating calli, (d) Plants shifted to Magentas

manipulation.

Somatic Embryogenesis: It is considered that all somatic cells of a plant are totipotent. However, all cells do not undergo embryogenesis in culture medium. Totipotency levels differ

from organ to organ of a plant depending on genotype. Results of our study show clearly that the varieties used hold different levels of embryogenic response on different media from different explants. Of the four varieties tested, comparatively, Swatl and Swatll showed greater embryogenic

response on media tested, but still that was not comparable with the embryogenic response of Basmati 370 or Basmati 385. Callus bore fine white hairs in case of Swatl, SwatlI and Dilrosh 97. Shoot growth with no callus formation was also observed. Root cluster accompanied with poor callus formation was observed in case of Pakhal on both media. This callus forming response of different rice varieties showed that these varieties behaved differently in callus formation on different media from different explants. Abe and Futsuhara (1986) also observed the varietal and genotypic variability of callus formation. It would be concluded from this study that these varieties needed further optimization of media and explant selection for *in vitro* tissue culture response.

Different explants of these varieties were used for tissue culture studies, irrespective of variety and media, scutellum showed greater callus formation. Root callus forming ability was also comparable with scutellum on both media. Two media LS 2.5 (LS medium supplemented with 2.5 mg/l 2,4-D) and N6 (N₆ medium supplemented with 2 mg/l 2,4-D) was used to observe the *in vitro* response of different explants of different varieties. N6 medium gave higher percentage of embryogenic calli than LS-2.5 medium. One explanation for this difference is that there are components, which have different amount in the two media. These components are in the form and amount of nitrogen and concentration of 2,4-D.

Regeneration of Rice: Plant regeneration is an essential requirement for plant transformation technology. The four varieties were investigated for regeneration ability by using its two explants scutellum and root on three different media. Both explants gave comparatively moderate regeneration. Plant regeneration from embryogenic scutellum calli was more efficient on RM medium containing sucrose 3 sorbitol 3% along with phytohormones NAA 1 mg/l and BAP 2 mg/l and casamino acids 0.25% solidified with 0.4% phytigel. Sorbitol increased the osmolarity of medium and resulted in better and efficient regeneration (Jain *et al.*, 1995). Another important factor was the use of high concentration of phytigel instead of agar. Furthermore, culture growth on the high osmolarity medium gave higher frequencies of plant regeneration and retained regeneration ability for longer periods than culture grown on the simple medium.

Age of explants is also important factor. It was found that calli of three week for both explants (scutellum and root

callus) gave better regeneration than two week old calli. From this study it was concluded that different varieties of rice respond differently in their inherited ability of embryogenesis and regeneration. Swatl variety gave the highest regeneration on (66.66% + 1.4%) RM medium, when scutellum derived calli was used. While the same variety gave highest regeneration (46.66 ± 0.66) when root derived calli was used on RM medium.

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