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Short Communication

Optimization of callus induction and callus multiplication in rice (*Oryza sativa* L.) landraces

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***In vitro* selection for abiotic stress tolerance in rice is one of the most common and reliable way for improvement of selection efficiency, but this requires standardized protocols. A protocol for tissue culture experiment, particularly callus induction, callus multiplication and plant regeneration were standardized from germinated juvenile rice seedlings. Plant explants were prepared from the junction portion of coleoptile and radical of 5 days old juvenile seedlings and induced to callus induction in callus induction medium. The generated callus tissues were sub cultured in callus multiplication media and plantlets were regenerated from sub cultured calluses. The standardized protocol is now routinely used for *in vitro* screening, micropropagation and transformation experiments.**

Key words: Rice, callus induction, callus multiplication.

Rice is the staple food for 65% of the population in India. Globally, rice accounts for 52% of the total food grain production and 55% of total cereal production. India is the second largest producer of rice (FAOSTAT Data base, 2006). For quick micropropagation, transformation and *in vitro* screening an efficient standard protocol is essential, particularly for callus induction, callus multiplication and plantlet regeneration. It has been reported that *indica* subspecies are more specific than japonica to tissue culture conditions and also for *Agrobacterium*-mediated transformation (Ge *et al*., 2006). Potentiality for callus induction and plantlets regeneration in culture condition depends on a number of factors, like genotype of the donor plants, physiological and biochemical status of the explants, composition and concentration of different ingredients of culture medium *etc.* Among these factors, genotypic difference is the most important one (Abe and Futsuhara, 1985, 1986). A number of reports have shown that most of the *indica* lines are less responsive to callus induction and regeneration as compared to *japonica* lines (Abe and Fursuhara, 1984, 1986; Reddy *et al*., 1994; Kavi Kishor and Reddy, 1986; Mikami and Kinoshita, 1988). Even not all the *indica* subspecies have the equal potentiality for *in vitro* responses (Hartke and Lorz, 1989; Ozawa and Komamine, 1989; Peng and Hodges, 1989; Oinam and Kothari, 1993; Seraj *et al.*, 1997; Khanna and Raina, 1998). In addition to the composition of culture media, the concentrations of plant growth regulators also influence the process of callus induction, callus multiplication and somaclonal variation. For somatic culture, MS medium (Murashige and Skoog, 1962) is most widely used as basal medium both for *indica* and *japonica* varieties. A number of

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reports have shown that the N6 (Chu *et al*., 1975) and LS (Linsmaier and Skoog, 1965) media gave an additional response for some *japonica* rice lines. Most of the published reports (Mandal *et al*., 2003; Ozawa *et al*., 2003) on tissue culture experiments have been carried out on specific improved rice lines. Present work was carried out on a less popular, drought avoiding traditional rice lines of South Bengal for which no previous reports are available on *in vitro* response. At present the standardized protocol is routinely used in other rice lines for *in vitro* screening, micropropagation and transformation with high efficiency.

Materials and Methods

Plant materials

The plant material selected for present experiment was *Oryza sativa* var. Gorah, a less popular indigenous rice line of South Bengal. The most significant property of this line is drought avoiding property, which is very much significant in development of stress tolerant rice genotypes.

Preparation of explants

Five mature healthy grains were placed in a beaker and surface sterilization was done with 20% commercial sodium hypochlorite solution for 10 minutes. The sterilized grains were thoroughly rinsed with sterile distilled water for 3-5 times to remove all possible traces of sodium hypochlorite and then soaked in sterile distilled water for 24 hours. All these steps were carried out under sterile condition. After 24 hours of soaking the grains were incubated for germination in an incubator.

Induction of callus

After three days small tissues from the swollen junction of radicle and coleoptile were excised and inoculated in callus induction medium (CIM) containing MS basal salts with 2.0 mg/litre (9mM) 2,4-dichlorophenoxyacetic acid (2,4-D). The inoculated culture tubes were placed in tissue culture room at 25±2°C with a 16/8 h light/dark cycle (light intensity 20 µmole m^{-2} s^{-1}, cool white fluorescent TLD Philips 18W/33). Callus tissues were started to generate within 7-9 days of one month incubation.

Multiplication of callus

After a month, induced calluses were transferred to callus multiplication medium (CMM) containing normal MS medium with half concentration of 2, 4-D of the CIM and incubated in dark for 21 days. The cross section of the generated callus tissues were observed under microscope and the amount of generated callus tissues were estimated.

Sub culturing of callus

After completion of 21 days duration, callus tissues from individual culture tubes were collected and placed in sterile petriplates under aseptic condition for dissection using sterile surgical blades. The dissected calluses were inoculated in freshly prepared MS medium with 4.52 mM of 2, 4-D and incubated for 21 days in dark.

Regenration of plantletes

After 21-28 days of incubation, individual calluses were transferred to regeneration medium containing basal medium (MS medium) supplemented with 0.5 mg/litre naphthaleneacetic acid, 2 mg/litre Kinetin and 0.50 mg/litre 6-benzyladenine. The calluses were inoculated for a month under 16/8 h light/dark cycle as mentioned earlier.

Transfer of the plantlets to artificial soil

Within 20-30 days of incubation, the small green plantlets were developed and 20 days old small plantlets were taken out from the culture tube, the adhered medium from roots was removed and transplanted in small plastic glass filled with artificial soil.

Results and Discussion

This study was a part of investigation on *in vitro* screening for drought tolerance and agro-infection on a selective rice genotype

for which no earlier reports are available. The plant tissues originated from different stages of *in vitro* propagation are given in fig.1. The transverse section of callus tissues showed high degree of homogeneity which is very much essential for successful *in vitro* micropropagation. A good number of reports are available on different Basmati genotypes and HYV rice lines, but reports on rice landraces and wild relatives are very limited. This standardized protocol is now routinely used on a number of different land races and wild rice relatives. This standardized protocol may also be used for artificial seed production which is very important for germplasm conservation.

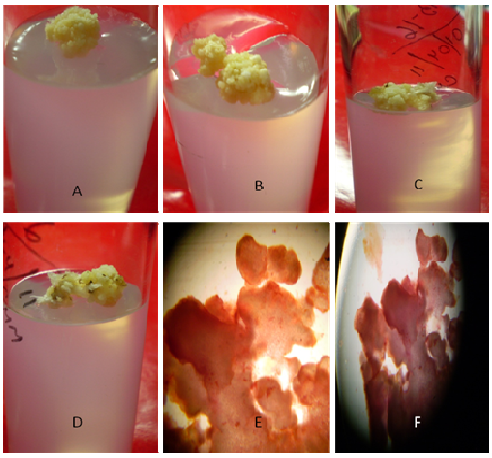

Figure: 1. A to D Callus formation, E and F Microscopic view of transected callus tissue (stained with safranin)

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