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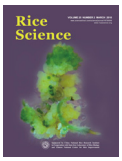

Sprouted Sorghum Extract Elicits Coleoptile Emergence, Enhances Shoot and Root Acclimatization, and Maintains Genetic Fidelity in *indica* Rice

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Abstract: The high growth-stimulating effect of plant extract has urged the plant biotechnologists to use natural supplements in the culture media instead of synthetic phytohormones. We advocated the effect of sprouted sorghum extract (SSE) on emergence, *in vitro* acclimatization, and genetic fidelity in coleoptile derived callus of *indica* rice variety ADT36. The use of SSE with Murashige Skoog medium efficiently acclimatized the root and shoot apical systems. A higher mat and seminal roots (3.4 g biomass) with an efficient shoot primordium elongation were observed with an increase in the concentration of SSE. Seeds treated with SSE medium showed higher germination and earlier coleoptile maturation about 48 h compared to untreated seeds, and there was a higher expression of *eEF-1α* with an increase in coleoptile length. B5 medium was effective on inducing embryogenic and nodular callus from 3-day-old coleoptile with 3.0 mg/L 2,4-dichlorophenoxyacetic acid and further proliferated effectively with 0.8 mg/L kinetin with a fresh weight of 180 mg. Highly significant regeneration was observed with combination of 2.5 mg/L 6-benzylamino purine and 3.0 mg/L α-naphthaleneacetic acid. The metabolic and genetic profiles of *in vitro* and directly cultivated plants were the same, examined through Fourier-transform infrared spectroscopy, random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) and R-ISSR (combination of RAPD and ISSR) markers, respectively, and thus confirming the significant efficacy of the SSE incorporated medium. Disarmed T-DNA was transformed to coleoptile derived callus through *Agrobacterium tumefaciens* LBA4404 and confirmed by *GUS* assay. The T-DNA integration was confirmed by DNA blot analysis using DNA from transient *GUS*-expressed explants. Thus, SSE can be used as a natural and organic supplement for organogenesis and efficient acclimatizations of shoot and root apical meristems in regenerated plants.

Key words: callus induction; coleoptile; *Oryza sativa*; random amplified polymorphic DNA; inter-simple sequence repeat; regeneration; tissue culture

The advents of new and modern techniques are taking the plant molecular biology to a next generation of research. The ultimate success of transgenics relies on the efficiency of the tissue culture and regeneration protocol (Koetje et al, 1989; Raemakers et al, 1997; Sanyal et al, 2005; Dabul et al, 2008; Mishra and Rao, 2016; Grant et al, 2017). Tissue culturing of a plant involves important aspects like explant selection (source and nature of the explant), media optimization (composition of the medium for organogenesis), and *in vitro* cultivation, and the maintenance of a controlled environment (Thorpe, 2007). Firstly, the

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explant selection wherein different methods/protocols have been proposed to induce calli from mature seed (Abe and Futsuhara, 1985, 1986; Wang et al, 1987; Azria and Bhalla, 2000), root part (Abe and Futsuhara, 1985; Mandal et al, 2003), coleoptile segment (Oinam and Kothari, 1995; Sahrawat and Chand, 2004) and leaf base (Ramesh et al, 2009). The seed texture, growth, germination and coleoptile formation play pivotal roles in signaling the plant growth which relies on the phytohormones and vitamins incorporated in the media (Miransari and Smith, 2014). Secondly, the tissue culture media wherein many basal media have been proposed and are being used, such as MS (Mdrashige and Skoog) medium for *in vitro* cultivation of *Nicotiana tabacum* (Murashige and Skoog, 1962), LS medium modified from MS with variation in thiamine-HCl (Linsmaier and Skoog, 1965), B5 medium for suspension culture from root cells (Gamborg et al, 1968), and N6 medium for another culture from flowers (Chu, 1988). These media have micro- and macro-elements that help in the metabolism of a plant. Vitamins, nutrients and sugar sources are added externally for efficient *in vitro* cultivation. The third important aspect is regeneration and acclimatization of the *in vitro* cultured plants. Though the synthetic hormones are efficient in regeneration and organogenesis, frequent occurrence of somaclonal variation remains a deterrent. In the recent decades, researchers are very keen to use more natural and organic supplements so as to generate somatic cells or clones without any somaclonal variations. The tissue culture of plants can be improved by incorporation of natural organic supplements which varies from species to genotypes (Thorpe, 2007). Different extracts have been used to culture the explants *in vitro* with or without plant growth hormones (PGHs) as medium supplements, such as coconut water to induce explant development (Imani et al, 2009), yeast extract as a key source for inositol and thiamine (Robbins and Bartley, 1937), potato extract as a source of carbohydrate, amino acid and vitamin like VC, VB_1 and VB_{12} (Storey, 2007), cytokinin compound from tomato xylem sap (van Staden and Menary, 1976), and casein enzyme hydrolysate as a natural supplement for micro-element, phosphate, calcium, vitamin and 18 different amino acids (George et al, 2008).

Here, we proposed sprouted sorghum extract (SSE) as a natural supplement instead of synthetic PGHs in the basal medium to enhance organogenesis and *in vitro* acclimatization. The tissue culture protocols for *Oryza sativa* varieties are well established, and they are certainly a model system to study any preliminary approach or genetic makeover to compare with other plant species. *indica* rice variety ADT36 was developed by Tamilnadu Rice Research Institute (TRRI), Aduthurai, Tamilnadu, India and chosen for *in vitro* cultivation. It is a widely cultivated rice variety along the Cauvery delta districts of Tamilnadu, India (Geethalakshmi et al, 2011). ADT36 is resistant to biotic stress like rice blast, brown planthopper, and leaffolder (Rani et al, 2007). The rice is resistant to drought and high temperature. It has a cultivation period of 110 d and 1000-grain weight of 20.6 g which makes it as a suitable crop for cultivation and commercial benefit. Genetic manipulation of this variety could enhance its characteristic traits to resist or adapt to stressful conditions. We proposed an empirical approach for tissue culture using SSE for rapid coleoptile induction, reproducible and efficient acclimatization of shoot and root apical meristem in ADT36.

MATERIALS AND METHODS

Sprouted sorghum extract preparation and rice seed germination

Wild type sorghum seeds were procured from a Farm Aid Service in Madurai, India. The sorghum seeds were washed with distilled water, and weighed 10 g in each 100 mL distilled water in a conical flask. Then, the flask was kept in an orbital shaker at 25 ºC overnight with 100 r/min to germinate. The germinated sorghum seeds were blot-dried and crushed to a fine paste by mortar and pestle, and were made up to 100 mL with distilled water. The extract solution was then filtered with Whatman No. 1 filter paper to remove the residues, and was again made up to 100 mL with distilled water. The ADT36 seeds were surface sterilized (Krishnan et al, 2013), and inoculated to germinate on various media.

Callus induction from different explants

Different explants such as mature seed, leaf base, coleoptile, and root from the germinated rice seedlings were checked for callus induction on B5, LS, MS and N6 media, supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/L). The cultures were incubated in dark at (26 ± 2) ºC for 28 d. The mature,