CALLUS INDUCTION AND PLANTLET REGENERATION ABILITY OF SELECTED SRI LANKAN TRADITIONAL RICE CULTIVARS (ORYZA SATIVA L.) IN CARYOPSES CULTURE

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ABSTRACT

A total of forty Sri Lankan traditional rice varieties (Oryza sativa L) were examined for their callus induction, growth and regeneration ability at Laboratory of Agricultural Biology, Faculty of Agriculture, University of Ruhuna. Surface sterilized rice seeds (caryopses) were cultured on four different callus induction media supplemented with 1.5, 2.5, 3mg/l 2,4-Dichlorophenoxy acetic acid (2,4-D) and 0.1mg/l 6-benzyladenine (BA) on Murashige and Skoog’s (MS) basal medium. Proliferated calli were regenerated on 4 different regeneration media supplemented with 1.5, 2, 2.5, 3mg/l BA with 0.1mg/l naphthalene acetic acid (NAA). Eighty seven point five % of rice cultivars were able to produce callus on callus induction medium and among them 12.5 % of rice cultivars regenerated shoots. 100% callus induction was recorded by 75% of tested rice cultivars on 2.5mg/l 2,4-D supplemented to MS basal medium while the callus induction percentages were 30% and 32.5% in 1.5mg/l 2,4-D and 3mg/l 2,4-D supplemented media respectively.

Callus growth of rice cultivars showed significant difference with the cultivar and medium. By the 4th week of callus induction maximum diameter (> 2cm) of callus recorded by rice cultivar Herath on 2.5mg/l 2,4-D + 0.1mg/l BA + MS basal medium. The regeneration ability of rice cultivars varied from 0 % to 100% while the highest regeneration was recorded in rice cultivar Herath on regeneration medium supplemented with 2.5mg/l BA and 0.1mg/l NAA. Callus induction varied from 0% - 100% and significantly high callus formation was offered in Dik wee, Herath, Puchchai Perumal and Rathu wee. Sudu bala wee, Kahata wee (long grain), Sudu heenati, Kahata samba and Muthumala failed to initiate callus on any of the medium. Significantly highest shoot regeneration percentage was found in Herath (100%) while Mas samba, Seedevi, Dostara heenati and Rathel were also categorized in to the same DMRT (Duncan’s Multiple Range Test) group with Herath. The highest number of shoots (8 shoots/callus) was recorded in Herath compared to other varieties (0.1 -8 per explant). There was a significant interaction between callus induction medium and rice cultivar as well as regeneration medium and rice cultivar.

Key words: Oryza sativa L. Traditional rice, Callus, Regeneration

INTRODUCTION

Rice is a cereal crop that belongs to family Poaceae and is of tremendous economic importance. It is a staple food for a large human population in the world. It is feared that world population would be around 10 billion by 2050. Thus more food will be required to feed the human population. This will be in the backdrop of diminishing cultivated land. Studies are underway to increase yield as well as quality of rice.

Sri Lanka is considered as a valuable repository of crop germplasm and agro-biodiversity. It has a rich treasure of rice genes and over 2,800 varieties have been recorded in the country. These varieties show great adaptability to a wide range of climatic and soil conditions as well as pest and disease problems. They also exhibit variation in grain size and quality, some with medicinal properties and fragrance and others that are used for cultural and ritual reasons also show differences in maturity period. (http://goviya.com/agro-biodiversity2.htm).

A diet of traditional rice three times a day gave our ancestors all the energy they needed to carry out a multitude of heavy tasks, from ploughing paddy fields to chopping firewood, to walking several miles daily and still have energy to spare. Ailments such as heart attack, high blood pressure or diabetes were almost unheard of. Different historical records re-
revealed that traditional rice varieties were revered for their nutritional and medicinal properties.

Regeneration from callus was achieved many years ago in Japonica varieties (Nishi et al., 1973). The potential for callus formation and regeneration has been reported to be a varietal characteristic. An efficient callus induction and proliferation followed by regeneration in Indica rice is still poses a major problem for genetic manipulation through innovative approaches (Toki, 1997). While it has been possible to obtain high callus induction, proliferation and plant regeneration frequencies in Japonica rice varieties, the success for reproducible fertile plant regeneration has been limited in Indica rice varieties so far (Kyozuka et al., 1988; Raman et al., 1994). As a result, progress towards the transfer of useful genes into Indica rice has been slow. Many factors have been examined to improve the frequency of callus induction and plant regeneration in rice.

Organogenesis of callus tissues depends upon plant species, type of explant from which the callus was derived, age of callus tissue and composition of the nutritional medium. Another important factor is nature and level of various growth regulators. Although there has been some success in plant regeneration from rice tissue culture, the protocol so developed is not applicable to all the cultivars of rice. Therefore, a suitable method has to be developed for local rice varieties. Forty traditional rice varieties were selected for the present study since they are localized varieties to Sri Lanka and no callus induction and regeneration has been reported earlier.

**MATERIALS AND METHODS**

Seeds of forty traditional rice varieties (Oryza sativa L) were collected from Plant Genetic Resource Centre, Ganoruwa, Peradeniya and the experiment was conducted at the laboratory of Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna in 2011. Seeds were cultured on Murashige Skoog’s (1962) basal medium supplemented with 3% sucrose with different concentrations of 2,4-D (1.5, 2.5, 3.0 mg/l) and 0.1 mg/l BA. pH of the medium was adjusted to 5.6 and solidified with 0.6% agar. Phyto-hormones were added to the medium before pH adjustment. The medium was autoclaved at 15 psi for 15 minutes at 120°C. Experiment was done according to the complete randomized design with 10 replicates and was repeated three times. Sub culturing was done after 2 weeks and repeated two times. Cultures were kept in 16 hours cycled fluorescent light cooled incubators with temperature regulated at 25±1°C.

Proliferated Calli were grouped according to callus diameter; Group 1- <0.5 cm, Group 2- >0.5 – 1.0 cm, Group 3- >1.0 – 1.5 cm, Group 4- >1.5 – 2.0 cm, Group 5. > 2 cm and Group 6- no callus formation. Calli were transferred to MS based regeneration medium supplemented with different concentrations of BA (1.5, 2.0, 2.5, 3.0 mg/l) and 0.1 mg/l NAA. Number of shoot developed in each culture tube was counted after 30 days in the regeneration medium. Data was analyzed by Student Newman-Kuells Means Separation Test of SAS (SAS Institute, Cary, NC, 1995).

**RESULTS AND DISCUSSION**

There was a significant difference in callus induction ability of traditional rice cultivars in different callus induction media (Fig. 1A and B). By the second week of culturing rice cultivars Dik wee, Herath, Pachchai Perumal and Muthumala initiated callus induction while Kolokuttu, Yakada wee, Polayal, Sivuru wee and Manamalaya started callus induction within 4 weeks . Among these varieties significantly highest callus induction was recorded on MS medium supplemented with 2.5, mg/l 2,4 –D regardless of rice cultivars. Rice cultivars Sudu bala wee, Kahata wee (long grain), Sudu heenati, Kahata samba and Muthumala were unable to produce calli on any of the medium.

Among callus producing cultivars the highest callus induction percentage (87.5%) was recorded by Herath on MS + 2.5 mg/l 2,4 – D of the selected traditional rice varieties maximum callus formation was observed in Dik wee, Herath, Pachchai Perumal and Muthumala and...
these were the cultivars responded early for callus induction.

Callus induction as well as regeneration potential was affected not only by genotype and the type of explant but also by composition of the culture medium including plant growth regulators, and by culture conditions. This is in agreement with Rueb et al. 1994 who have reported that particular genotype and type of explant are important factors for the successful embryogenic callus induction and regeneration of rice plants.

The highest regeneration percentage (100%) was recorded by Herath on regeneration medium supplemented with 2.5mg/ml BA and 0.1 mg/ml NAA and this was the rice cultivar which recorded the earliest callus induction and greatest callus proliferation (100%) but there was no positive relationship between time to take for callus initiation or callus proliferation ability with regeneration.

Among tested regeneration media significantly high regeneration was recorded on MS basal medium supplemented with 2.5mg/l BA regardless of rice cultivar. There was no relationship between the callus induction medium and regeneration medium. Though Herath recorded the highest callus production and regeneration percentage among all the tested rice cultivar, other rice cultivars that recorded higher callus proliferation in callus induction medium recorded lower regenerated plantlets in the tested regeneration media.

The successful application of plant tissue culture techniques for crop improvement requires suitable plant regeneration methods. In rice, there are many reports on successful plant regeneration from calli derived from different explants (Nishi et al. 1973; Yamada et al. 1967a, b; Furuhashi and Yatazawa 1964), including inflorescences (Kavikishore and Reddy, 1985). However, the use of tissue culture to propagate traditional rice in Sri Lanka is limited, since regeneration can be obtained only in a limited number of selected traditional rice varieties. This communication summarizes among 40 traditional rice cultivars tested, plantlet regeneration was observed in Herath, Mas samba, Seedevi, Dostara heenati, Rathel, Suduru Samba.
CONCLUSION

Application of 2,4-D at to the basal callus induction medium significantly increased callus initiation as well as callus proliferation of rice regardless of the cultivar. Other concentrations of 2,4-D in MS basal medium acted differently on rice cultivars for callus proliferation.

In future studies, 2mg/l 2,4-D supplemented to the MS basal medium with 0.1 mg/ml BA can be recommended for most of the rice cultivar for highest callus proliferation and the MS based regeneration medium supplemented with 2.5mg/l BA and 0.1mg/l NAA as regeneration medium. However but the best combination of callus induction and regeneration media is yet to be found for rice cultivars as the regeneration from callus induction medium was not elaborated in this study.

REFERENCES

Abe T and Y. Futsuhara 1985 Efficient plant regeneration by somatic embryogenesis from root callus tissue of rice (Oryza sativa L.) Plant Physiol. 121: 111-118


Kavikishor PB and Reddy GM 1986 Regeneration from long term cultures and selection of useful mutants in rice. In: Gene structure and function in higher plants (ed).


