INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 2, p. 144-150, 2014

RESEARCH PAPER OPEN ACCESS

The effect of plant growth regulators on embryogenic callus induction and regeneration from coleoptile in rice

Hamideh Ghobeishavi, Ebrahim Dorani Uliaie*, Seyed Siamak Alavikia, Mostafa Valizadeh

Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Key words: Rice, somatic embryogenesis, regeneration, plants growth regulators, coleoptile.

http://dx.doi.org/10.12692/ijb/5.2.144-150 Article published on July 28, 2014

Abstract

To achieve a repeatable tissue culture system is the first successful step in the genetic engineering of plants. For obtaining this objective in this study potential of coleoptile explant and tow genotype for regeneration were examined. Effect of different treatments on callus induction and regeneration from coleoptile explant was investigated. Significant difference was observed between two cultivars in callus induction and somatic embryogenesis. The plant regeneration was influenced by the genotype as well as composition of the medium. The high frequency plant regeneration was achieved from both cultivars in medium containing 2 mg/L kinetin plus 0.5 mg/L NAA and medium containing 2 mg/L BAP plus 0.5 mg/L kinetin. Regeneration percentage of *Neda* variety (32.39%) was higher than *Nemat* variety (20/95%).

* **Corresponding Author:** Ebrahim Dorani Uliaie ✉ dorani@tabrizu.ac.ir

Introduction

Rice is one of the most important cereal crop and staple food source for more than half of the world's population (Vega *et al.*, 2009). Rice provided 49% of the calories and 39% of the protein in the humans diet (FAO, 2012). Rice consumers are increasing at the rate of 1.8 % every year without any systematic increase in production (Karthikeyan *et al.*, 2009). It is estimated that rice production has to be increased by 50% by 2025 to feed the ever-growing world population (Khush and Virk, 2000). With the development of plant molecular biology and genetic engineering, its transformation has become one of the core issues in molecular breeding. First requirement for the successful application of biotechnology in crop improvement is to have efficient plant regeneration from cultured cells and tissues. (Evangelista *et al.*, 2009). Efficient plant regeneration from different explants is a decisive step for genetic improvement of crops through Biotechnology (Christou, 1997). Several factors including plant growth regulators, explant, culture conditions and plant genotypes affect rice somatic embryogenesis and subsequent plant regeneration (Deo *et al.*, 2010). These factors have different interact with explant and plant genotype. Plant regeneration in rice via organogenesis or embryogenesis has been reported from different explants, such as root (Hoque and Mansfield, 2004), mature embryo (Jose Pons *et al.*, 2000), leaf (Ramesh *et al.*, 2009), immature embryo (Noori-Delawar and Arzani, 2001), and protoplast (Jelodar *et al.*, 2002). In general, there is a few reports in plant regeneration through coleoptile explants in cereal, and plant regeneration from coleoptile tissue of *Poa pratensis* L.(Ke and Lee, 1996), durum wheat (Benkirane *et al.*, 2000) and rice (Akter and AL-Forkan, 2010) have been reported. Little reports is available on the totipotency of coleoptile in *Indica* rice (Oinam and Kothari, 1995).The coleoptile explant can be obtained easily at any time in in vitro culture condition through germinating the seeds. The coleoptile provides easy materials for use in gene transfer experiments and in comparison with the immature embryo (Nehra *et al.*, 1994) and immature inflorescence (Barcelo *et al.*, 1994) can be used more easily in transformation experiments and are available in each season. Therefore the purpose of the study described in this paper was to attempt to develop a reproducible plant regeneration system in vitro from coleoptile explants of selected Iranian cultivated rice genotypes for future genetic transformation studies.

Material and method

Explants preparation

Two important rice (*Oryza sativa* L.) cultivars (cv. *Nemat* and cv.*Neda*) were provided from Rice Research Institute of IRAN (Rasht) and dehusked manually. The dehusked healthy seeds were surface-sterilized by immersion in 70% ethanol for 90 sec., followed by 5 minutes shaking in sterile distilled water and then 20 minutes shaking in 50% Clorox (Sodium hypochlorite 2/5%) and finally rinsing three times in sterile distilled water. Sterilized seeds were kept for germination on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) devoid of growth hormones, supplemented with 30 g/L sucrose and 7g/L agar. The pH of the medium was adjusted to 5.7–5.8 prior to autoclaving. Cultured seeds were incubated for the first 2 day in dark and then transferred under diffuse cool white fluorescent light (50 m mol m^{-2} s^{-1}) with 16/8 h photoperiod at 25–27°C. 1-cm long coleoptile segments were dissected by cutting the base and the tips of coleoptiles from five days old seedlings.

Callus induction and somatic embryogenesis

Coleoptile segment were cultured on MS medium supplemented with 2/5 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) for callus induction and cultures were kept in dark for 4 weeks at 26 ± 2°C (Fig 1A). After 4 weeks, the percentage of callus induction was calculated as follows = (The number of callus induced ÷ The number of incubated explants) × 100%. for developing somatic embryos calli were subcultured in same media except that the level of 2,4-D was reduced to 0/5 mg/L. explants were kept under 16/8 h light/dark photoperiod and subcultured every three weeks. After 6 weeks, the embryogenic callus frequency were scored as percentages (The number of embryogenic calli ÷ The

number of calli) × 100%.

Plant regeneration

After 10 weeks of culture initiation embryogenic calli were transferred to new MS medium supplemented with six different composition of plant growth regulators (table 1). Each treatment was repeated three times in 10cm petridishes which consisted of 8 embryogenic callus.

After 45 days regeneration frequency were calculated as follows = (The number of plantlets regenerated ÷ The number of embryogenic callus) × 100%.

Statistical analysis

The experiment was carried out in factorial with a completely randomized design with 3 replications. After 6 weeks the mean number of regenerated explant for each treatment was determined. Statistical analysis performed using SPSS for Windows release 16.0. Differences between means were scored with Duncan's multiple range test.

Result and discussion

Callus induction from coleoptile explants

Callus induction started after 7 days from cut end of coleoptile segments on MS medium containing 2/5 mg/L 2,4-D. after 2 weeks callus induced in entire length of the coleoptile explant (Fig 1B) in both cultivar. Nodular embryogenic calli were observed clearly 20 days after inoculation. Embryogenic calli (Fig 1D) were cream in color, compact and globular while non embryogenic calli were observed whitish, watery, friable and in some cases have rhizogenic structure.

Table 1. Different regeneration medium compositions.

	Treatment			
Medium	BAP(mg/L)	Kin(mg/L)	NAA(mg/L)	IAA(mg/L)
1 (MS)	-	-	-	-
2	2	-	-	-
3	2	0/5	-	-
4	4	-	-	-
5	4	-	-	0/5
6	-	2	0/5	-

Statistical analysis of callus induction percentage of two cultivar was done by using t-test and variety mean square was significant at 5% level that showed presence of significant differences between two cultivars in callus induction. The percentage of callus induction in *Neda* and *Nemat* cultivars was 58% and 48%, respectively. This result showed Callus induction and regeneration controlled through genetically factors which are in nucleus or cytoplasm and genotype is a determining factor in response to tissue culture even *between varieties* within *species (Kuroda et al., 1998). Akter an AL-Forkan (2010) were observed* significant differences between different rice varieties *in terms of callus induction, somatic embryogenesis and regeneration. In this experiment callus induction occur at both cut end and in some segments developed along the entire length of coleoptile. Oinam and Kothari (1995) and Akter and AL-Forkan (2010) reported callus induction at both basal cut end and entire length of rice coleoptile segments.*

Somatic embryogenesis of coleoptile explant

four weeks old good extended calli were transferred to MS medium where 2,4-D concentration decreased to 0/5 mg/L and the percentage of somatic embryogenesis was calculated after 6 weeks (Fig 1C). Statistical analysis by using t-test showed significant differences between two cultivars at 5% level. The percentage of somatic embryogenesis in *Neda* and *Nemat* cultivars was 30% and 18/5%, respectively.

The calli were transferred to new medium with less concentration of 2,4-D for somatic embryo induction. The high concentration of 2,4-D increased callus induction but decreased frequency of embryogenic calli. Sahrawat and chand (2001) were reported highest frequency embryogenic calli was achieved when embryogenic calli at first were transferred on MS medium containing 9 µM 2,4-D and 2/32µM kinetin and then sub cultured on MS medium supplemented with 2/25 µM 2,4-D, 2/32 µM kinetin and 490 µM l-tryptophan. Somatic embryogenesis were increased by transferring the calli from medium containing high level of auxin to low level auxin (Viertel and Hess, 1996). In other cereals also have been emphasis to use low concentration of 2,4-D for embryo developing (Viertel and Hess, 1996).

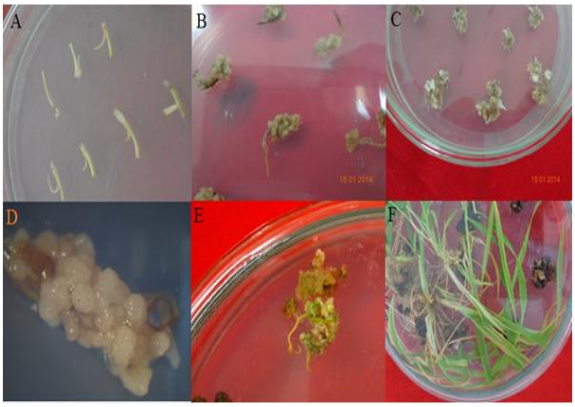

Fig. 1. Embryogenic callus induction and plant regeneration from coleoptile segment of rice. A) coleoptile segment cultured on MS medium supplemented with 2/5 mg/L 2,4-D. B) Nodular and embryogenic calli inductionfrom cut ends and *along the entire length of coleoptile*segment. C) somatic embryos induction on MS medium containing 0/5 mg/L 2,4-D. D) Embryogenic callus. E) Shoot formation. F) Green multiple shoots.

The effect of different treatment of plant growth regulators on regeneration from coleoptile explants

Plant regeneration was achieved through somatic embryogenesis and organogenesis from coleoptile derived calli. After transfer the embryogenic callus to the medium containing various concentration of plant growth regulators regeneration of plantlet started. Shoot bud structure was observed after 20 days from embryogenic calli mass on regeneration medium (Fig 1E). These shoot buds further developed and multiplicated and differentiated into green multiple shoots (Fig 1F). Some of the embryogenic calli in different regeneration medium getting brownish or blackish and finally died. Statistical analysis showed that two cultivar and different regeneration treatments have significant differences on regeneration percentage of rice plantlet at 5% and 1% level, respectively. The percentage of regeneration in *Neda* cultivar (32/39%) was higher in compared to *Nemat* cultivar (20/95%).

Six type of culture medium that used for regeneration were scored with Duncan's test and in three separate a, b and c groups. The highest frequency of regeneration was achieved in mediums containing 2 mg/L kinetin plus 0.5 mg/L Naphthylacetic acid (NAA) and medium containing 2 mg/L 6-Benzyleaminopurine (BAP) plus 0.5 mg/L kinetin, respectively. And no significant difference was observed between these two hormonal treatments (Fig 2).

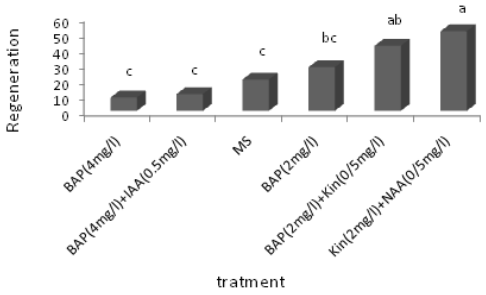

Fig. 2. Effect of different treatments on rice regeneration.

culture medium composition, ratio and type of plant growth regulators and explant are important factors in regeneration potential of plants. To stimulate organogenesis of embryogenic calli, adjust the hormonal ratio of auxin and cytokinin is necessary. The sensitivity of embryogenic callus to this various hormonal ratio refer to endogenous phytohormone levels which is also affected by genotype factors (Khanna and Raina, 1998).

here we observed potential of callugenesis and regeneration in rice is dependent on genetic structure and nutrition of culture medium. This result are in agreement with Panday *et al.* (1994) who reported

success in tissue cultures generally is dependent on the medium constitute, plant growth regulators, variety and the interaction between genotype and the medium.

High frequency Embryo regeneration was achieved in media supplemented with Kin. Kinetin via affected mitosis, cytokines, protein synthesis, lignin biosynthesis, vascular differentiation and differentiation of mature protoplast promoted proliferation and regeneration of callus (wan *et al*., 1988). Saharan *et al.* (2004) were reported high regeneration in *Indica* rice varieties was obtain on culture medium containing 2 mg/L kinetin and 0/5 mg/L NAA. Because cytokinin will causes constitute of meristematic tip from which the shoot primordium arises (Verma *et al*., 2011). Medium with BAP or BAP plus Indole-3-acetic acid (IAA) have not significant different with hormone free medium in regeneration. But plant regeneration of calli obtain from rice coleoptile was achieved on MS medium supplemented with BAP and IAA (sahrawat and chand, 2001). BAP in combination with NAA have been reported to facilitate the regeneration of *Indica* rice calli (karthikeyan *et al*., 2009). Akter an AL-Forkan (2010) also were reported high regeneration percentage of rice coleoptile explant obtained on MS medium containing 2 mg/L BAP with 0/5 mg/L kinetin.

Conclusion

In conclusion, the coleoptile tissue is an easily available explant and can be obtainedeasily by germinating seeds at any time in year. (Sahrawat and Chand, 2001). Coleoptile explant have capacity of embryogeniccalli formation and plants regeneration for a wide range of varieties (Benkirane *et al*., 2000). In our experiment efficient plant regeneration frequency of two cultivar was abtained by using coleoptile as an explant, therefore coleoptile tissue can be used as target tissue for direct gene transfer through *Agrobacterium* or biolistic methods.

Reference

Akter P, AL-Forkan M. 2010. Assessment somatic embryogenesis and plant regeneration from coleoptile and root tissue of Jhum rice (Oryza sativa L.). Indian Journal of Agricultural Research **44,** 88-95. http://dx.doi.org/10.5958/j.0976-058X

Barcelo P, Hagel C, Becker D, Martin A, Lorz H. 1994. Transgenic cereal (*Tritordeum*) plants obtained at high efficiency by microprojectile bombardment of inflorescence tissue. Plant Journal **5,** 583-592.

Benkirane H, Sabounji K, Chlyah A, Chlyah, H. 2000. Somatic embryogenesis and plant regeneration from fragment of immature inflorescences and coleoptiles of durum wheat. Plant Cell, Tissue and Organ Culture **61,** 107-113. http://dx.doi.org/10.1023/A:1006464208686

Christou P. 1997. Rice transformation: bombardment. Plant Molecular Biology **35,** 197-20 http://dx.doi.org/10.1023/A:1005791230345

Deo PC, Tyagi AP, Taylor M, Harding R, Becker D. 2010. Factors affecting somatic embryogenesis and transformation in modern plant breeding. The South Pacific Journal of Natural and Applied Sciences **28,** 27 – 40. http://dx.doi.org/101071/SP10002

Evangelista FC, Aldemita RR, Ungson LB. 2009. Callusing and regeneration potential of rice (*Oryza sativa* L.) genotypes towards the development for Salt tolerance. Philippine Journal of Science **138,** 169-176.

FAO. 2012. FAOSTAT (available at: www.faostat.faoorg/

Hoque EMd, Mansfield JW. 2004. Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of *indica* rice genotype. Plant Cell, Tissue and Organ Culture **78,** 217-223.

Jelodar NB, Davey MR, Cocking EC. 2002. Plant

regeneration from cell suspension protoplasts of two Iranian japonica rice cultivars and ploidy level of regenerated plants. Journal of Agricultural Science and Technology **4,** 141-149.

Jose Pons M, Marfa V, Mele E, Messeguer J. 2000. Regeneration and genetic transformation of spanish rice cultivars using mature embryo. *Kluwer Academic* Publishers. Printed in the Netherland **114,** 117-122.
http://dx.doi.org/10.1023/A:1003941913609

Karthikeyan A, Pandian STK, Ramesh M. 2009. High frequency plant regeneration from embryogenic callus of a popular *indica* rice (*Oryza sativa* L.). Physiology and Molecular Biology of Plants **15,** 371-375.
http://dx.doi.org/10.1007/s12298-009-0042-6

Ke S, Lee CW. 1996. Plant regeneration in Kentucky bluegrass (*Poa pratensis* L.) via coleoptile tissue cultures. Plant Cell Reports **15,** 882-887.
http://dx.doi.org/10.1007/BF00231580

Khanna HK, Raina SK. 1998. Genotype × culture medium interaction effects on regeneration responses of tree *indica* cultivars. Plant Cell, Tissue and organ culture **52,** 145-153.
http://dx.doi.org/10.1023/A:1006032303195

Kuroda S, Kato H, Ikeda R. 1998. Heterosis and combining ability for callus growth rate in Rice. Crop Science **38,** 333-336.
http://dx.doi.org/10.2135/cropsci1998.0011183X003800040008x

Khush GS, Virk PS. 2000. Rice breeding: Achievement and future strategies. Crop Improvement **27,** 115-144.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15,** 473- 479.
http://dx.doi.org/10.1111/j.13993054.1962.tb08052.x

Nehra NS, Chibbar RN, Leung N, Caswell K, Mallard C, Steinhauer L, Baga M, Kartha KK. 1994. Self fertile transgenic wheat plant regenerated from isolated scuteller following microprojectile bombardment with two distinct gene construct. Plant Journal **5,** 285-297.
http://dx.doi.org/10.1046/j.1365313X.1994.05020285.x

Noouri-Delawar MZ, Arzani A. 2001. Study of callus induction and plant regeneration from immature embryo culture in rice cultivars. Journal of Science and Technology of Agriculture and Natural Resources **4,** 57-72.

Oinam GS, Kothari SL. 1995. Totipotency of coleoptile tissue in *Indica* rice (*Oryza sativa* L. cv. CH1039). Plant Cell Reports **14,** 245-248.
http://dx.doi.org/10.1007/BF00233642

Pandey SK, Ramesh B, Gupta PK. 1994. Study on effect on genotype and culture medium on callus formation and plant regeneration in rice (*Oryza sativa* L.). Indian Journal of Genetic **54,** 293-299.

Ramesh M, Murugiah V, Gupta AK. 2009. Efficient in vitro plant regeneration via leaf base segments of *indica* rice (*Oryza sativa* L.). Indian Journal of Experimental Biology **47,** 68–74.

Saharan V, Yadav RC, Yadav NR, Chapagain BP. 2004. High frequency plant regeneration from desiccated calli of *indica* rice (*Oryza sativa* L.). Africa Journal of biotechnology **3,** 256-259.

Sahrawat AK, Chand S. 2001. High frequency plant regeneration from coleoptile tissue of indica rice (*Oryza sativa* L.). Invito Culture and Developmental Biology **37,** 55-61.
http://dx.doi.org/10.1007/s11627-001-0011-4

Tyagli AK, Khurana JP, Khurana P, Raghuvanish S, Gaur A, Kapur A, Gupte V, Kumar D, Ravi V, Vij S, Khurana P, Sharma, S. (2004). Structural and functional analysis of rice

genome. Journal of Genetics **83,** 79-99.

Vega R, Vasquez N, Espinoza AM, Gatica AM, Melara MV. 2009. Histology of somatic embryogenesis in rice (*Oryza sativa* cv. 5272). Revista de Biologia Tropical **57,** 141–150.

Verma D, Joshi R, Shukla A, Kumar P. 2011. Protocol for in vitro somatic embryogenesis an regeneration of Rice (*Oryza sativa* L.). Indian Journal for Experimental Biology **49,** 958-963.

Viertel K, Hess D. 1996. Shoot tips of wheat as an alternative source for regenerable embryogenic callus cultures. Plant Cell, Tissue and Organ Culture **44,** 183-188**.** http://dx.doi.org/10.1007/BF00048522.

Wan Y, Sorensen EL, Liang GH. 1988. The effect of kinetin on callus characters in alfalfa (Medicago sativa L.). Euphitica **39,** 249. http://dx.doi.org/10.1007/BF00037103