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Short Article

Different Growth Factors Supplement and Growth Response in Callus Culture of Rice (Oryza Sativa L.)

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Abstract

Callusing response of rice IR – 20 (explants – seed embryos) was assessed in MS- medium supplemented with 2,4-D, kinetin, Choline chloride, Glycine and Choline chloride with glycine separately. It was assessed by performing protein estimation, cell number counting and on the basis of fresh and dry weight of the callus. The callusing response was high (80%) in MS-medium supplemented with choline chloride. The protein content was more in MS-medium supplemented with glycine. The number of cells present in the callus raised on MS-medium supplemented with 2,4,-D and Kinetin was higher as compared to other growth factors supplemented media Somatic embryos were observed in the callus culture raised on MS+2,4-D and kinetin. Different protein levels were measured in the callus culture raised in MS-medium supplemented with 2,4-D, Kinetin, Choline chloride and Glycine.

Keywords: MS medium, Callus, Supplemented, Embryoss

Introduction

Rice has been cultivated for more than 7000 years as a major food crop and currently feeds more than 50% of the world population (Izawa and Shimamoto 1996). Tissue culture has played important role in rice improvement n recent years. Rice cultivated is economically and agriculturally essential in the south-east Asian countries. In India, 83% of cultivated area is under food crops, of which rice is covers about 32%. IR -20 was developed from a cross of IR-262-24-3 and TKM-6 at IRRI, Philippines. This cultivar is weakly photosensitive. Rice is rich in carbohydrates and some of the vitamins. This rice variety (IR-20) is one of the ergonomically important cultivar. It is predominantly grown in tropical and subtropical regions of India. The genome of rice is diploid and the genome size is 5×10^8bp. Of this, almost 50% is single copy DNA. Rice also has a large gremplasm collection. In this present experiments we planned to study effect of different types of plant growth regulators and amino acids on somatic embryos and embryogeniccalli induction.

Materials and method

Rice seeds (Oryzasativa L. Indica cv IR – 20) was collected from Tamil Nadu Agricultural University, Coimbatore. The dehusked seeds were put in a beaker containing 70% ethanol and incubated for 3 min. Then washed with sterile double distilled water thoroughly for five times and followed by $HgCl_2$(0.1%) treatment for 5 min. Then washed with sterile double distilled water for six times. The rinsed seeds were inoculated on the MS medium (Murashige and skoog, 1967) containing different growth supplements 1. (MS+2,4,-D 5µm Kinetin 5µm (control) (MS I), 2. MS + 2,4-D 5µm + Kinetin 5µm + choline chloride 10µm (MS II), 3. MS + 2,4-D 5µm + Kinetin 5µm + glycine 25µm (MS III) and 4. MS + 2,4-D 5µm + Kinetin 5µm + choline chloride 5µm + glycine 25µm (MS IV). Using sterile forceps, this was

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immersed in 70% ethanol and flamed and an average of 5-7 seeds were inoculated per culture tube.

Culture tubes with the mature seed explants were kept in dark at 25 ± 2ºC for 25 days. Good callusing response was noted after a month in those MS media based formulations. The inoculated primary culture was transferred on to a fresh culture medium after 25 days. The composition of the sub culturing medium is same as that of callus induction medium. Sub culturing was done under sterile condition in front of the flame, culture were incubated under light intensity of 2500 1ux at 25 ±2ºC for 21 days and subsequently used for protein estimation, checking somatic embryos and cell number counting.

Results

Callusing response of the rice cultivar IR – 20 (explanted – seed embryos) was assessed in MS medium supplemented with 2,4-D 5 µm + Kinetin 5 µm in combination with choline chloride and glycine each of these separately and together. Callusing response was based on the basis of fresh weight, dry weight, protein content (Lowery's method) and cell number counting of the callus. MS-medium supplemented with four different growth supplements were used for the development of callus from the seed embryos of IR – 20. It was observed that the frequency of callus formation was high in MS II (Table 1).

The fresh and dry weight of the callus was weighted after 15 days of culture in the supplemented media. It was found that both the fresh and dry weight of the callus was more in MS II medium compared to other combinations (Table 2). Protein content of the callus was done

15th day after callusing by following lowry's method. The amount of total protein was also high in the embryogenic callus tissue raised in MS II medium (Table 3). Effect of these four different growth supplements on callusing response was also checked by counting the cell numbers (15 days after callusing). Haemocytometer was used for counting the cells. It was found that the number of cells were high in the tissues raised in MSI medium (Table 4) on the basis of cells per gram fresh weight..

In one set of experiment, occurrence of somatic embryos in the callus culture was assessed. The control cultures viz., MS medium with 2,4-D and kinetin grown callus was microscopically observed for the somatic embryos. In those culture, several dense clusters of cells (pro-embryos) as well as typical embryo stages viz. Globular – embryo was observed (Fig not shown). In a recalcitrant culture of a rice spreads, the occurrence of somatic embryo under the experimental conditions of the present study is quite on interesting one. Further study is needed to characteristics the "embryogenic factors " in those cultures.

SDS-PAGE (8-18% gradient) of protein extracted from rice calli grown on the above four different types of MS supplement medium. Value of the major polypeptides found in the calli grown on four different types of MS supplemented medium. During 15th and 40th day of culture, in MS III and MS IV medium proteins were observed and it was completely absent in other type of medium.

Discussion

In general growth hormones such as kinetin and 2,4-D are routinely used for the induction of callus. In the present study, attempts were made to find out the effect of growth supplements such as choline chloride and glycine on callus induction in a rice cultivar IR-20. The MS – medium was supplemented with 2,4-D + kinetin (control) and choline chloride + glycine or choline chloride and glycine separately.

Callusing response was found to be 65-80% in the different MS-medium with growth factors supplements. But the callusing response was high (80%) in MS-medium supplemented with choline chloride 5µm. These results showed that choline chloride plays a role in induction of callus. Likewise the fresh and dry weight of the callus as weighed on 15 day after callusing showed similar effect due to choline supplementation. It was also observed that the fresh weight accumulation of callus tissue was more in MS – medium supplemented with choline chloride.

Effect of these growth supplements on the protein content and cell number of the callus tissue was also assessed. The amount of protein was more in callus tissue raised in MS – medium with glycine on a per gram fresh weight basis. This result indicates that glycine an amino acid supplement might play a role on callus formation. On the contrary, the number of callus present in the callus raised in MS – medium supplemented with 2,4-D + kinetin was more, indicating that kinetin, a cytokinin, played a major role in cell division. Like our result Nhut et al., (2000) and Ahn et al ., (1996) were also observed the significant increased formation embryogeniccalli by supplementation of 2,4-D to the cultures and till date the transgenic rice cultivars have also been produced through 2,4-D supplementation shoot (Liangcayi et al ., 1993, Shiping et al ., 1996, Rajinder et al ., 1996, Hamid et al ., 1996.). With the above results we conclude that the embryogenic callus induction nature was highly increased by the supplementation of choline chloride and it will definitely leads to rice gremplasms improvement.

Table 1.Callusing response due to various growth supplements in rice

Medium code	Medium	Frequency of Callus formation (%)	Type of the cells
MS I	MS + 2,4-D + KIN	65	Calli slow growing
MS II	MS + 2,4-D + KIN + Choline chloride	80	Yellowish calli Highly proliferated
MS III	MS + 2,4-D + KIN + Glycine	70	Yellowish calli Highly proliferated
MS IV	MS + 2,4-D + KIN + Choline chloride + Glycine	65	Yellow calli Slow growing

Table 2.Fresh and dry weight of callusing responses of four different types of MS medium

Medium code	Medium	Growth parameter on 15th day		Growth parameter on 40th day	
		Fresh wt. (g)	Dry wt. (g)	Fresh wt. (g)	Dry wt. (g)
MS I	MS + 2,4-D + KIN	0.3	0.075	0.7	0.17
MS II	MS + 2,4-D + KIN + Choline chloride	0.5	0.1	1.0	0.7
MS III	MS + 2,4-D + KIN + Glycine	0.3	0.05	0.6	0.08
MS IV	MS + 2,4-D + KIN + Choline chloride + Glycine	0.2	0.06	0.5	0.09

Table 3.Effect of growth factor supplements on protein of callus of rice

Medium code	Medium	Total proteins on 15th day Mg/g. Fr.Wt	Total proteins on 40th day Mg/g. Fr.Wt
MS I	MS + 2,4-D + KIN	17.54	25.00
MS II	MS + 2,4-D + KIN + Choline chloride	10.58	22.20
MS III	MS + 2,4-D + KIN + Glycine	47.26	54.24
MS IV	MS + 2,4-D + KIN + Choline chloride + Glycine	11.98	21.5

Table 4.Effect of growth factor supplement on cell number of callus cultures of rice

Medium code	Medium	Cell number on 15th day g.Fr.Wt	Cell number on 40th day g.Fr.Wt
MS I	MS + 2,4-D + KIN	$3.6x\ 10^5$	3.9×10^5
MS II	MS + 2,4-D + KIN + Choline chloride	2.8×10^5	3.1×10^5
MS III	MS + 2,4-D + KIN + Glycine	1.6×10^5	1.8×10^5
MS IV	MS + 2,4-D + KIN + Choline chloride + Glycine	2.3×10^5	2.5×10^5

Conflict of Interest: None

Reference

1. Ahn Io, Bui VL, Gendy C, TranThanh Van K (1996). Direct somatic embryogenesis through thin cell layer culture in *panax ginseng*. Plant cell tissue org cult. 45:237-243 Hamid R, Shuuji y, Kinya T, Kokichi H (1996). Transgenic plant production mediated by *Agro bacterium* in Indica rice. Plant cell Rep 15:727-730.
2. Izawa T, Shimamoto K (1996). Becoming a model plant : the important of rice to plant science. Trend plant sci 1(3) : 95-99.
3. Liangcai L, Rongda Q, Alexander DK, Claude F, Roger NB (1993). An improved rice transformation system using the biolistic method. Plant cell Rep : 12 : 250-255.

4. Murashige T, Skoog F (1962). A revised medium for a rapid growth and bioassay with tobacco tissue culture. Physiol plant. 15 : 473-479.
5. Nhut D.T, B,V.Le and K.T.T. Van (2000) stomatic embryogenesis and direct shoot regeneration of rice *(Oryzasativa L.*) using thin cell layer culture of apical meristematic tissue. Plant Physiol. 157: 559-565.
6. Rajinder KJ, Sunita J, Baiyan W, Ray W (1996). Optimization of biolistic method for transient gene expression and production of agronomically useful transgenic Basmati rice plants. Plant cell Rep. 15: 963-968.
7. Shiping Z, Lili C Rongda Q Philippe M, Roger B, Claude F (1996). Regeneration of fertile transgenic indica (group 1) rice plants following micro projectile transformation of embryogenic suspension culture cells. *Plant cell Rep*. 15: 456-469.

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