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Callus Induction and Plant Regeneration of Rice from Embryo (*Oryza sativa* L.)

S.K. Reddy and Omar H. Obaid
Cytogenetics, Tissue Culture and Molecular Biology Lab.
Department of Botany, Osmania University, Hyderabad-7, Telangana,
E-mail : skreddybot@gmail.com

In this present study two varieties of indica rice (Tulasi and Jarava) obtained from Indian Institute of Rice Research (IIRR). The seeds were sterilized with 70% ethanol and 0.01%$HgCl_2$ for 5 minute for each time. The matured embryo ex-plants were transferred in to MS media supplemented with 2,4-D (2,4-dichlorophenoxi acetic acid) and Kn (Kinetin) concentration 1.0, 2.0 and 0.1, 0.5 mgl^{-1} respectively. The plant regeneration was achieved by transferring callus on to MS media containing with 1.0 mgl^{-1} NAA (Naphthalene acetic acid) and BAP (6-benzyl amino purine) 1.0, 2.0 and 3 mgl^{-1}.

Best response of callus induction was observed for both varieties (Tulasi and Jarava) MS media supplemented with 2.0 mgl^{-1} 2,4-D and 0.1 mgl^{-1} Kn . Out of these two varieties best calli obtained for Tulasi .Then the callus was transferred to MS medium consisting of 1 mgl^{-1} NAA and 3 mgl^{-1} BAP showed maximum plant regeneration. Jarava best regeneration was obtained with MS medium supplemented with 1 mgl^{-1} NAA and 2 mgl^{-1} BAP. The variety Jarava preformed best plant regeneration than the Tulasi. The regenerated plants were acclimatized in pot sand culture.

Keywards : Regeneration, Sterilization, Hardening.

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family poaceae, it comes in third position after wheat and maize, rice as other important crops increasing demand for it (Roy,1985) production of this crop reach to 749.1 million tons in 2014-2015 (FAO, 2015). Rice is the staple food more than half of the world's population. Globally rice is cultivated in 156 million hectare (Mha) with an average productivity of 4.12 tonnes/hectare (t/h). India stands first in area and second in production.

After 35 year problem will be worsen because the world's population will grown more than half the current population in same time rice production facing many problems like salinity, water scarcity and diseases with traditional production programs, the production will decrease and its will not be possible to fill people of rice needed (Shanthi *et al.,* 2010). In this present investigation variety Tulasi short duration for 100 day upland rain fed variety. The other variety jarva long duration (150 day) and saline tolerant one developed for costal area cultivation are used.

For this problem must choose correct way biotechnological approaches, the application of plant tissue culture in combination with conventional breeding methods That the production of callus provides an opportunity to create genetic variation for the development of the qualities desired (Monirul *et al.,* 2005) than proportional and increase production as well as resistance environmental non- controlled that the work on the level of the cell and tissue means a small space dominated by high accuracy and that this process be a team limited work in a short time and regardless of the seasons Agriculture. Induction callus from different explants (embryos mature and immature and other explants) dependent on conditions used in agriculture, the type of plant, growth regulators, media, so the balance in growth regulators in media lead to different percentage from callus induction.

In this primary study of tissue culture techniques achieved in Cytogenetics, tissue culture and molecular biology laboratory Department of Botany, Osmania University. The two indica varieties of Tulasi and Jarava, cultured on MS media (Murashige and Skoog, 1962) with different concentrations of auxins and cytokinins were used for induction of callus and for regeneration of plants.

MATERIAL AND METHODS

Explant source : In current experiment mature embryos of two indica rice varieties (*Oryza sativa* L.) were used as explant for induction callus, the rice varieties (Tulasi and Jarava) were obtained them from (Indian Institute of Rice Research) IIRR.

Explant sterilization : The mature embryos were dehausked in the laminar air flow cabinet and the explants immersion in 70% ethanol for 5 min. and treated with mercuric chloride (0.01)% for 5 min.. After that explants were rinsed 4 - 5 times with sterile distilled water and processes for explants sterilization described by many researchers.

Media preparation : MS powder was used for both experiments for induction of callus and regeneration of plants, this media prepared as (Table 1) supplemented with vitamins as (Table2) and 500 mg proline, 1 mg myoinositol, sucrose 30 g/l with combination of two kinds of growth regulators 2,4-D and kn (1,2) mg/l and (0.1, 0.5) mg/l respectively for induction callus, same protocol used for plant regeneration media except change growth regulator NAA and BAP 1 mg/l and (1,2,3) mg/l respectively .The pH of the media was adjusted at 5.5 - 5.8 by using pH meter. The media was solidify with 7.5 g/l of agar and cooked until boiling, after that poured in to the culture tubes, bottles and autoclaved at 121ºC, 15Lbs for 15 minute.

Seed incoulation : After removal of the seed coat and sterilization, one seed is inoculated for each tube and this process conducted in sterile environment, and then it is transferred to culture room at, 25ºC ± 2 and dark condition.

Plant regeneration : After 28 day dark culture the callus transferred to the plant regeneration media, then the callus chopped to parts almost similar diameter of 1 cm as much as possible and then distributed by 5 pieces for each bottle and then transferred to the culture room at, 25 ºC ± 2 and period of light 16 hours per day.

Plant hardening : To acclimatization plant to normal environment added 50 ml of water in a bottles containing plantlets fixed on media with the change of water daily and opening the lid gradually after first week of transplant into the soil covered with plastic bag in pot with irrigation cascade keeping high moisture around the plant, making wholes in bags gradually increase during a week after that removed the bag.

RESULTS

The aim of the experiment was to find best combination to induction callus and regeneration of plants from two varieties of rice was recorded as fresh weight, calli were observed after one or two weeks of mature embryo inoculation onto MS media, the calli white and pale yellow increase in growth value until four weeks.

Noted that the highest rate of production of callus was in a variety Tulasi, which differed significantly from the Jarava, fresh weight of callus in variety Tulasi 792.3 mg and in case of Jarava 625.2 mg. Lowest rate for the production of callus in Tulasi was 521.2 mg, while the lowest rate of callus in variety Jarava 191.6 mg (Table.3, Fig 1). The difference in the concentration of 2,4 -D had a significant effect in the production of callus was found that media, with 2 mgl^{-1} 2,4-D it was better than media with 1 mgl^{-1} of 2,4-D in both the varieties, as well as in this experiment found that addition of Kn impact on the quantity and quality of callus product. But an increase in the concentration of the Kn affected negative impact on the amount of callus development and media supplemented 0.1 mgl^{-1} of Kn is more efficient to increase the amount of callus comparison with 0.5 mgl^{-1}, the difference was very clear in both varieties Jarava and Tulasi (Table 3, Fig 1&2) increase in callus amount and more in case of transfer to another media.

Table 1 : Murashige & Skoog medium.

Ingredients	mg/l
Potassium nitrate	3040.00
Ammonium nitrate	825.00
Calcium chloride.$2H_2O$	440.00
Potassium phosphate monobasic	170.00
Manganese sulphate.H_2O	16.90
Molybdic acid (sodium salt).$2H_2O$	0.25
Zinc sulphate.$7H_2O$	8.60
Copper sulphate.$5H_2O$	0.025
Cobalt chloride.$6H_2O$	0.025
Ferrous sulphate.$7H_2O$	27.80
EDTA disodium slat.$2H_2O$	37.30

Table 2 : Vitamins and other chemicals.

Ingredients	mg/l
Myoinositol	100
Nicotinic	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Glycine	2.0
Sucrose	30000
Agar	7500

Table 3 : Mean weight (mg) of callus induction on MS medium containing different concentrations of growth regulators 2, 4-D and Kn for two verities Tulasi and Jarava.

2,4-D mg/l	Kn mg/l	Tulasi	Jarava
1	0.1	557.5	320.9
	0.5	521.5	191.6
2	0.1	792.3	625.2
	0.5	789.4	409.2

Table 4 : Regeneration of plants from callus with phytohormones.

Growth regulators combinations	Tulasi	Jarava
NAA $1mgl^{-1}$ + BAP $1mgl^{-1}$	+	+
NAA $1mgl^{-1}$ + BAP $2mgl^{-1}$	++	++
NAA $1mgl^{-1}$ + BAP $3mgl^{-1}$	+	+++

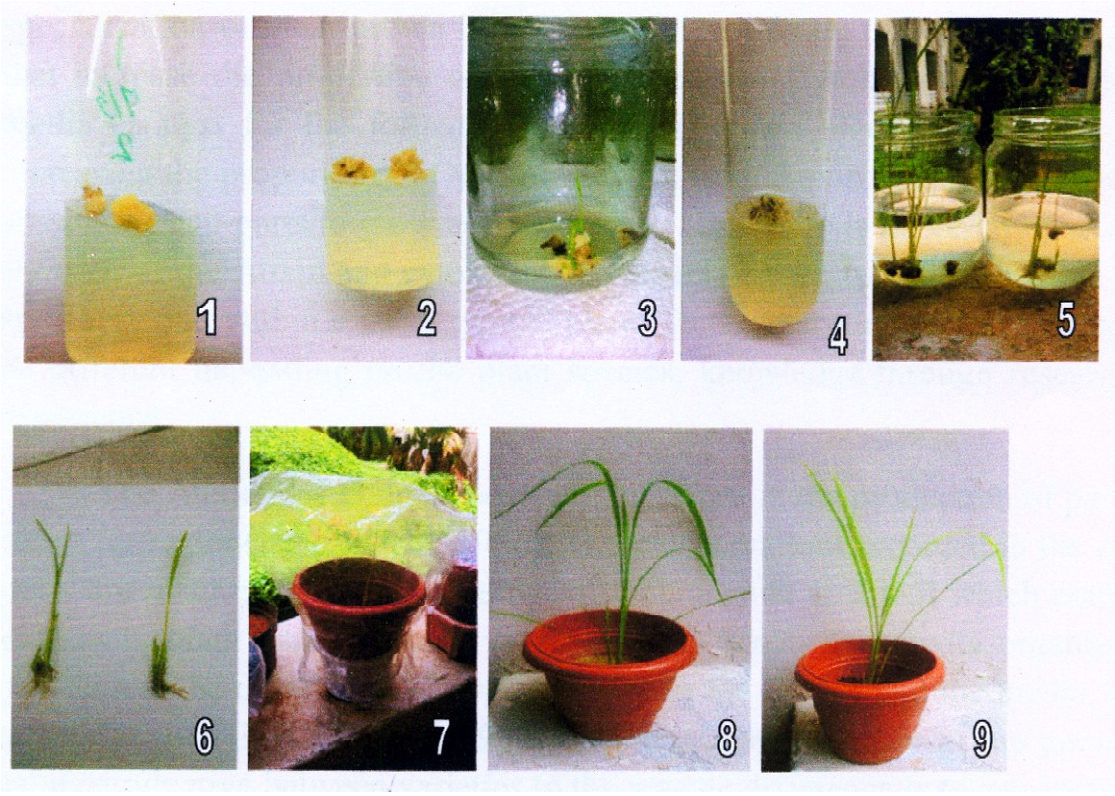

Figure : 1 to 9

1. **Callus inducation in variety Jarava when mature embryo on MS media with 2, 4-D and kn.**
2. **Callus inducation in variety Tulasi when mature embryo on MS media with 2, 4-D and kn.**
3. **Regeneration of plant from callus on MS media with BAP and NAA.**
4. **Callus on MS media free hormone developed roots only.**
5. **Tulasi and Jarava in hardening step with added water 200ml/bottles and change it daily in first week.**
6. **Hole plant with shoot and root for Tulasi and Jarava before transplant them to soil.**
7. **Plants in pot with plastic cover slotted it gradually until remove if after 1-2 week.**
8. **Jarava after hardening of 27 day.**
9. **Tulasi after hardening of 27 day.**

Regeneration of plants : MS media supplemented with growth regulators led to stimulate the formation shoots and roots at the same time, while the callus-free growth regulators did not produce shoots, but it was developed only roots (Fig 4).

Increase the concentration of growth regulators led to stimulate an increase in the shoots and roots growth in the number and quality, reaching a maximum in Tulasi at concentration 2 mgl^{-1} BAP and 1mgl^{-1} NAA but in for Jarava was the highest rate of growth in concentration 3 mgl^{-1} BAP and 1 mgl^{-1} NAA (Table 4, Fig 3).

DISCUSSION

The Auxin and cytokinins of plant hormones that have a very important role in the control of Plant growth process. These hormones have been used widely in plant tissue culture technology, and noted (Skoog and Miller, 1957) the existence of a relationship between Auxin and Cytokinins concentration in the media on the one hand and between the nature of the growth and specialization of plant grown in this part and media on the other. They found that increase the proportion of Auxin more from Cytokinnins lead to make a media catalyst for the formation of root cultivated plant parts which, while increasing the proportion of Cytokinnins more from Auxin make the media fit to stimulate plant parts to grow and form shoot. But there is no certain percentage for each of the Auxin and cytokinins in respect of each type of vegetable plants and every part within a same type of plant, Thus, determining these percentages be different depending on the plant species, knowledge through research and expenence.

The Auxin 2,4-D most powerful influence in the production of parts and callus formation (Abo-El-Nil, 1986). These observations recorded in the current study are in agreement with findings of (Go uranga *et al.,* 2015) which noted that success in plant tissue depends largely on the center and media organizations growth and the quality of the overlap between the quality and the media, which is similar to (Guo

and Cao, 1982) differences may be due in the amount of callus productive between varieties to the genetic difference between Jarava and Tulasi this is study agreement with what he found (Biswas and mandal, 2007) during his studies of several different varieties of rice in the production of callus between varieties each other.

In the current study selected several concentrations of growth regulators 2,4-D and Kn to produce callus in two varities (Tulasi and Jarava) high amuont of callus formed with 2,4-D $2mgl^{-1}$ + Kn $0.1mgl^{-1}$ (Table 3, Fig 1). The increase in the weight of soft callus may be due to a balance between Auxin and Cytokinnins with the internal balance of cells that work together to promote cell division (Dodds and Robert, 1995). The plant regeneration obtained, MS media supplemented NAA $1mgl^{-1}$ + BAP $2mgl^{-1}$ in two varities and with BAP $3mgl^{-1}$ henhaced regeneration is observed in varitey Jarva (Table 4, Fig 2). As the high concentration of it to reduce the proportion of lead and motivate a number of shoots and the reason for this is due to the inhibitory influence whes increasing the limit be appropriate because the increase in concentration caused accumulation there by increasing demolitions(Taiz and Zeiger, 2003).

CONCLUSION

The present regeneration studies are useful for the development of salt, drought tolerent plants and also useful for development of transgenic plants.

Competing interests : The authors declare that they have no competing interests.

Authors contributions : First author desigened the problem, second author carried out the experiment. Both the authors prepared the manuscript togather and approved the final manuscript.

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