

Effect of Genotype and Growth Regulators in Induction of Embryogenic Callus in Rice

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Rice is the primary source of food in many countries of the world and has conducted many studies in order to improve breeding and rice production, especially in the field of plant tissue culture. The aim of this study is to review some of the factors that influence the induction of callus from rice seeds. Of these factors are that genotype and explants source are important parameters in determining the success of rice plant regeneration in tissue culture. The other factor plant hormones, like animal hormones, are relatively small molecules that are effective at low tissue concentrations. The most generally used plant hormones in tissue culture are cytokinins and auxins. Cytokinins are derived from adenine and exert two immediate effects on undifferentiated cells: the stimulation of DNA synthesis and increased cell division. Still studies dealing with the induction of callus of rice and the factors influencing it, such as genotype and growth regulators is not sufficient to reach the best results and therefore it must conduct more studies on these factors in order to reach the best results that serve the breeding and improvement of rice production in quality and quantity.

Key words: Rice, callus induction, genotype and explants, Plant hormones.

Producing the rice (*Oryza sativa* L.) production considered as the main basis of salary for about one billion people all over the world and delivers the main food for about half of the world population (Dawe et al., 2011). It is expected that its demand enhances to about 35% by 2035 (FAO, 2002). By enhancing the world population, the world's requires 116 million tons more of milled rice (GRiSP, 2010). By considering the enhancements needs of rice, it is important to

improve rice production procedure which considered as a challenging task (Wassmann *et al.*, 2009). Conventional breeding considered necessary to increase rice but development is because of different obstacles (Wang *et al.*, 2005). Plant tissue culture techniques considered as important techniques for several academic activities, particularly regarding the stress resist, and also for several practical features of plant science. Presently, regarding a matured technology, the methods are not merely applied for the investigations in plant molecular biology and gene guideline but it is used to plant biotechnology and molecular breeding. Callus, considered as an initial component in plant tissue culture which defined

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as an disorganised tissue mass growing on solid substrate through the application of tissue culture technology, which can form from several parts of large intact plants (Mineo 1990). During callus diversity, merely those highest and peripheral cells in callus were stimulated into actively dividing cells, and the amount of overall difference typically relies on the hormone balance of the support medium and the physiological state of the tissue. Among several elements affecting callus induction and renewal, genotype and nutrient media structure considered as two major elements that decide the fate of *in vitro* raised cultures (Khanna and Raina, 1998). Plant hormones, like animal hormones, are relatively small molecules that are effective at low tissue concentrations. The most usually applied plant hormones in tissue culture are cytokinins and auxins. In this study, researcher reviewed different effects of the affecting factors for stimulating the developments and the establishment of callus.

Plant tissue culture

Plant tissue culture considered as the group of methods applied for maintaining or growing plant cells, tissues or organs below sterile circumstances on a nutrient culture intermediate of recognised composition. Plant tissue culture is usually applied for producing duplicates of a plant a technique named micropropagation. Plant tissue culture refers on the issue that several plant cells usually have the capability for regenerating a complete plant (totipotency) (Davey *et al.* 2005). *In vitro* cell and tissue-based scheme have marvellous capability in central investigation and for trade procedures like clonal propagation, genetic engineering and manufacturing valued metabolites (Neelakandan and Wang 2012). Improvement of tissue culture is directly associated to development in methods of protoplast, cell, tissue and organ culture, followed by the achievement in renewing entire plants out of the cultured plant resources (Mustafa *et al.* 2011). The method has progressed fast during to several years because of the widespread researches about the challenges associated to fundamental and practical features of plants. Information of tissue culture has donated importantly for knowing the issues related to development, metabolism, diversity and morphogenesis about the plant cells (Kärkönen *et al.* 2011). Plant tissue culture

considered as the countless concentration to molecular biologists, plant breeders and manufacturers. The approaches of tissue culture were applied as a significant assistance for the old approaches of plant development (Germanà 2011; Bhatti and Jha 2010). They were applied to produce genetically adapted greater clones, ex-situ conservation of germplasm, pathogen free plants and (Loyola-Vargas and Ochoa-Alejo 2012; Sharry *et al.* 2011). The benefits presented by tissue culture in agriculture and overall plant biotechnology have well been witnessed by many research labs and industries. Somaclonal irregular strains from callus cultures are additional equipment to old breeding for producing the drought stressed resilient plants (Larkin and Scowcroft 1981; Dix 1993; Ashraf 1994). Presentation of the related genotype in *in vitro* selection relies on its ability to *in vivo* cultures, mainly to callus introduction and embryogenic callus manufacture. Investigations identifies several types that genotype influences plant in *in vitro* culture reaction.

Definition of calluses

Plural calluses or calli refers to disorderly parenchyma cells resultant from plant tissue (explants) to be used biological investigations and biotechnology. Regarding the plant biology, callus cells refers to the cells which usually covers the plant wound. Callus development is induced from plant tissues afterward surface sterilization and plating onto *in vitro* tissue culture medium. Plant development regulators like auxins, cytokinins, and gibberellins, are usually complemented into the medium for initiating callus formation or somatic embryogenesis. Callus origination was described regarding many plant taxonomic divisions (Collin & Edwards, 1998).

Impact of several affective factors regarding the Rice callus induction

Several factors can influence plant tissue culture like, genotype (Huang *et al.* 2002; Glowacha *et al.* 2010; Park *et al.* 2011), exogenous and endogenous hormones (Jiménez 2005; Barreto *et al.* 2010; Sun and Hong 2010; Huang *et al.* 2012).

Genotype and explants source

According to several reports, genotype and explants source considered as significant elements for identifying being successful for recreating rice plant in tissue culture (Liu and Lia,

1991). Zafar *et al.* (1992) achieved to the maximum callus formation in basmati rice cv.370 on MS medium accompanied with 2.0mg/l of 2,4-D. Though, somatic embryogenesis was attained by consuming MS medium added with 2mg/l from per 2,4-D and Kin. According to several reports, several factors usually influences plant renewal amount in rice; genotype, improvement step of calluses in the explants, hormonal arrangement of the medium, carbohydrates basis, fractional drought or water stress persuading treatments and other medium extras (Saharan *et al.*, 2004). Ilahi *et al.* (2005) mentioned that callus of a local diversity of rice (*Oryza sativa* L. cv. Swat-II) was made and the occurrence of callus induction was investigated on modified MS medium by using different combinations of 2,4-D and Kin, on the other hand, they stated that by adding tryptophan to several mixtures of auxins and cytokinins, the embryogenic callus mass would be increased and also callus would be effectively multiplied on MS accompanied with 1.0 mg/l of Kin and 0.5 mg/l of NAA. Khatun *et al.* (2010) reported that genotype dependence has significant role for different plant tissue culture activities and studied on callus induction and renewal potentials of twenty five rice cultivars through *in vitro* micropropagation and another culture.

Plant hormones

Plant hormones, alike animal hormones, are usually minor molecules which are operative at low tissue concentrations. The most frequently used plant hormones in tissue culture refers to cytokinins and auxins. Cytokinins are usually extracted from adenine and exert two instant properties on undifferentiated cells: by stimulating DNA synthesis and improved cell division (Ting 1982). Auxin and cytokinin are key elements of shoot diversity in callus culture (Skoog *et al.* 1965; Pernisova *et al.* 2009; Su *et al.* 2009; Cheng *et al.* 2010; Vanneste and Friml 2009; Zhao *et al.* 2010). Additionally, although ABA refers to an inhibitor of plant development, when it acts with related PGRs, would have positive consequences on plantlet growth (Rai *et al.* 2011; Huang *et al.* 2012). In earlier investigations, endogenous auxin, zeatin and ABA were at high levels in extremely generable rice callus (Liu and Lee 1996; Huang *et al.* 2012). Auxin could be the most important element for monitoring cell diversity (Petrašek and Friml 2009;

Rademacher *et al.* 2012). In earlier investigation it is found that endogenous auxin levels in rice calli can play pivotal roles throughout shoot renewal (Huang *et al.* 2012). Though, the way endogenous auxin variations can influence the effects of generable calli induction and shoot renewal is still unidentified. Auxins are indole or indole-like mixtures that stimulate cell growth, especially cell elongation that has been known that plant hormones works in association to each other and not separately, in planta (Mineo 1990). In the initial stage of plant tissue culture growth, Skoog and Miller (1957) mentioned that by absorptions of auxin and cytokinin in media we can identify the type of organogenesis that happened in callus and tissue cultures. Present knowledge of the mechanisms of cytokinin and auxin action at molecular level and their synthesis sites and supplies through the plant causes large operation of endogenous cytokinin and auxin levels by both use of exogenous cytokinin and auxin (Davies 1987; Mohnen *et al.* 1990; Branca *et al.* 1991) or the appearance of cytokinin/auxin biosynthesis transgenes (Smigocki and Owens 1989; Aoyama and Chua 1997; Kunkel *et al.* 1999; Sakai *et al.* 2000; Werner *et al.* 2003), and description of single gene mutants (Sakai *et al.* 2001; Friml *et al.* 2002). However our knowledge about molecular information in cytokinin and auxin regulation in planta improved, our information about biochemical and molecular events underlying the cytokinin/auxin regulating callus diversity of tissue culture are very insufficient, Although considered information is vital for illuminating the framework of the intracellular signal transduction trail from the insight of cytokinin/auxin to transcriptional regulation of primary cytokinin/auxin-responsive genes in planta. Those observations happened in rice calli (Nishimura and Maeda 1977; Abe and Futsuhara 1985; Jones and Rost 1989; Mendoza and Futsuhara 1992; Sangduen and Klamsomboon 2001; Vega *et al.* 2009) regarding the observation of callus alignment, somatic embryogenesis, and plant renewal, but were no done in the initial steps of callogenesis, that considered as an essential subject as this is considered a crucial stage for producing embryogenic calli (Yusoff *et al.* 2012). Mentioned the possible procedure of endogenous phytohormone signaling and carbohydrate

metabolism through shoot organogenesis brought by osmotic stress in rice (*Oryza sativa* L. cv. Tainung 71) callus, that auxin could be considered as a key factor which affects osmotic prerequisite, carbohydrate metabolism and phytohormone signaling on shoot renewal (Ting Lee and Lii Huang 2014)

CONCLUSION

Rice crops are essential for the financial life of several people all over the world. During the recent decades, rice yields have developed into plant tissue culture and are applied for developing breeding and agriculture yields like rice. Callus is a mass of tissue influenced by unregulated development of different elements. From these factors and the genetic explants source, considered as a source of significant restrictions to determine the achievements of the regeneration of the rice plant tissue culture. Other related elements that influences the Induction of callus, are plant growth regulators (PGRs) which has significant character for cell development. Cytokinin/auxin plant hormones, that has significant role in developing and differentiating of cells callus. The results that serve the refinement and development of rice manufacture in quality and quantity. Different studies investigated about the induction of callus of rice and the elements affecting it like genotype and development regulators is not enough for obtaining best consequences and therefore, more studies should be conducted on these elements for achieving to optimum results that serve the breeding and development of rice manufacture regarding both quality and quantity.

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