

# INDIRECT REGENERATION OF *INDICA* RICE FROM MATURE SEEDS: THE CURRENT SCENARIO

## Abstract

Rice is a popular C3 crop that feeds half of the world population. The requirement for rice production is rising as a result of the expanding population. However, various adverse environmental conditions affect rice productivity. Additionally, improved nutritional value of rice may aid in reducing the evil of malnutrition. Hence, it is imperious to mend such varieties that are able to overcome all these limitations. The biotechnological intervention through genetic engineering is the most practical approach for creating such rice varieties. For this alteration of genetic makeup through genetic transformation, the embryogenic calli formation and green plantlets regeneration under *in vitro* conditions are the key phases. However, the response of *indica* rice to *in vitro* condition is exceedingly reliant on genotype and there is a lack of straight forward, effective common methodology. According to numerous reports, *indica* rice cultivars are more specialized than *japonica* kinds to *in vitro* conditions and to *Agrobacterium*-mediated genetic modification. In order to improve the circumstances and produce successful outcomes, several investigations on various *indica* rice genotypes have been carried out by many researchers. In the last few decades, there has been a remarkable advancement. The goal of this article is to thoroughly examine the indirect regeneration of rice from its mature seeds. This provides a thorough understanding of the essential conditions and recent progresses for *indica* rice regeneration leading to rice biotechnology.

**Keywords:** *Oryza sativa*; mature seeds; scutellum; embryogenic callus, indirect regeneration, somatic embryogenesis

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## I. INTRODUCTION

Rice (*Oryza sativa* L.) is an essential food crop, predominantly in Asian countries like China, India and Thailand. The requisite for rice production around the world is continuously increasing due to population and economic progression, along with other socio-demographic issues. For cases, food preference, lifestyle vicissitudes and urbanization. This expansion in demand motivates additional efforts in refining the value of prevailing rice cultivars and upturn the productivity [1]. The foremost objectives in rice varietal development include superior quality, high yield and resistance to abiotic and biotic stresses [2]. Two major subspecies of rice are *japonica* (short grained, sticky) and *indica* (long-grained, non-sticky). *Indica* types, which are only cultivated in tropical and subtropical climates account for above 80% of world's rice production and hence, occupy a unique position in agriculture. [3]. Rice has also arose as the model monocot cereal for studying genome organization, gene function and behaviour of transgenes [4], because of its lesser genome size (430 Mb) [5] and closer syntenic relationship with other cereals [6].

There has been tremendous evolvement in recent years for the development of essential agronomic traits of rice through biotechnological interventions. *Agrobacterium*-mediated genetic transformation is considered to be the utmost popular system of plant genetic manipulation among all the other methods [7, 8 and 9]. It is not only used for producing genetically modified crops but also for functional genomic studies through transgenic and cisgenic approaches [10 and 11]. An enormous amount of data is being published every year based on functional characterization of plant genome using gene overexpression, silencing and ectopic expression strategies [12]. Due to its accurate gene transmission, single integration of foreign DNA, low copy number and transfer of bigger DNA segments with distinct ends, this approach is also the most popular [13]. Therefore, a proficient transformation system is a prerequisite for routine laboratory experiments in rice [14]. However, a major setback in such experiments is low transformation efficiency, which directly or indirectly depends on an extensive array of factors. These comprise plant species, genotype, type of explant, maturity, pH of media, regeneration and co-cultivation conditions, plant growth regulators, antibiotics, temperature, light, *Agrobacterium* strain and cell density, gene construct, cell competence after wounding and control of *Agrobacterium* overgrowth [15, 16, and 17]. These factors are interdependent and arise from the different phases or steps of transformation.

All the available transformation techniques need a proper morphogenetic system to regenerate transgenic shoots from transformed cells. However, the establishment of such cultures frequently struggle with intensive laboriousness, loss of plant regeneration capacity and unwanted albino or non-fertile plant production [18 and 19]. Rice and maize are well known cereals to be amenable for *in vitro* culture and regeneration [20]. Most of the *Agrobacterium*-mediated genome engineering systems in *indica* rice comprise plantlet regeneration from transformed anthers, embryogenic calli and protoplasts [21]. The *indica* rice, however, is known to be less receptive to *in vitro* culture than the *japonica* sub-species [22]. Research using model types including Nipponbare (*japonica*) and PB-1, IR64 (*indica*) has proved this fact [14 and 23]. Additionally, it has been noted that popular *indica* genotypes including IR64, Pusa Basmati1, CSR10 and Swarna are less susceptible to somatic embryogenesis and regeneration [6]. The advance of friable and superior quality callus capable of rapid shoot regeneration is critical to the success of *indica* rice transformation

using indirect regeneration schemes. Identification of such calluses is difficult, and regeneration takes time. Prolonged tissue culture is associated with browning of callus and results in low regeneration frequency [1]. The genotype dependent response also limits local *indica* cultivars for genetic modification [24]. Moreover, due to photoperiodic sensitivity, rice inflorescences and immature embryos are accessible merely for a narrow period in a year [25].

The scutellum (mature embryo) derived calli are most common and preferable source of explant for transformation in *indica* rice. It has several advantages like independent availability of season, geographical location, ease of operation and lesser infection by pathogens [26 and 27].

Several studies reported the optimization of tissue culture media with respect to phytohormones, organic and salt concentrations on diverse *indica* genotypes to produce high quality embryogenic calli that eventually improves the *Agrobacterium* mediated transformation [28]. The phytohormones like auxin and cytokinin, those control cell differentiation and development, are usually added in tissue culture medium during transformation. The concentration, combination and relative proportions of these exogenous hormones influence the transformation efficiency largely [29]. In a broad sense, the rice transformation depends on three major factors: 1) An efficient tissue culture protocol amenable for transformation, 2) An *Agrobacterium*-mediated plant transformation system for smooth gene delivery, and 3) Reliability/validity of the transformation system.

## II. INDIRECT REGENERATION OF RICE THROUGH TISSUE CULTURE

Under certain circumstances, the differentiated tissues of plant return to a dedifferentiated state or unorganized cell aggregates known as calli. However, in response to plant growth hormone stimulus, these calluses form tissues to regenerate whole plants. This competence of regeneration is totipotency that depends on the genetic potential of plants. There is a great possibility of totipotency existing in all plant species, but it is challenging to ascertain the conditions required for its expression [30]. Kumar *et al.* (2016) [31] suggested that optimization of *in vitro* regeneration scheme is indispensable for obtaining transgenic plants from transformed calluses.

Various plant growth hormones, gelling agents and amino acids have been tested to increase callusing percentage, as a pre-requisite for selection and recovery of the cells carrying the transgenes [32]. Ghareeb *et al.* (2009) [33] recommended that the proliferation of callus is highly inclined to medium constituents and especially growth regulators, while callus development was independently influenced by genotype and medium. Moreover, the callus obtained from mature embryos of rice cultivars are acquiescent to multiple shoot formation, and competent for rice transformation studies. There are many callusing and regeneration media available that rice explants respond to. Nevertheless, the key variances in these media are the concentrations of phytohormones, macronutrients, sucrose [34]. Numerous references to the regeneration circumstances of *indica* and *japonica* rice varieties have been found in the literature, and it has been shown that adding amino acids, macronutrients, growth regulators and other media supplements improves shoot morphogenesis and overall plant regeneration. The aspects that intensely stimulate embryogenic callus induction and shooting in rice are described here:

- 1. Genotype and subspecies:** The host plant genotype is the most vital factor in callusing and regeneration of rice [35]. The *indica* cultivars have genotypic differences that make them resistant to transformation and are typically regarded to be reflective towards *in vitro* culture and poorly receptive to genetic modification [34]. The lack of studies detailing transgenic *indica* lines may serve as more evidence that *indica* types are challenging to convert, despite the fact that the earliest accounts on rice transformation were accessible since the late 1980s [36]. Yan *et al.* (2010) [37] observed the regeneration frequency of 3.6-87.5%, 9.2-59.5% and 17.2-43.2% for *indica*, *japonica* and hybrid rice, respectively from mature embryos.
- 2. Type of explants:** Identification of acceptable explants to create embryogenic calli under proper culture conditions has been a continuing effort to increase callus production [38 and 39]. Due to their high regeneration ability, immature embryos are frequently employed as explants for callusing and genetic modification in a number of graminaceous species [40]. The immature embryo preparation is tedious and labour exhaustive, since it requires a complex and pricey greenhouse for continuous supply. On the other hand, the straightforward use of mature embryos like a donor for embryogenic callus has been shown to be effective in plant transformation [41]. In rice, mature seeds are mostly utilized for callus induction. Mature seed are effortlessly stocked and utilised, avoiding the demand for growing plants, and surpassing the necessity of sampling, i.e. plant growth period [29]. Rice seeds are additionally competent to callogenesis and somatic embryogenesis as equated to nodes or tips [42].
- 3. Plant growth regulators:** Plant hormones or growth regulators are crucial to plants. They promote the development of embryonic structure and the generation of callus during plant tissue culture [43]. Skoog and Miller first proposed the theory of hormonal balance in 1957 after learning that auxin and cytokinin might control morphogenesis and development of plant [44]. Plant endogenous hormones control gene expression of tissues, which affect metabolism and lastly conclude the initiation, conservation and manifestation of embryogenic prospective of cells [45].

The most popular growth regulator in cereals for obtaining embryogenic calli is 2, 4-dichlorophenoxyacetic acid (2, 4-D). In order to start and maintain embryogenic callusing in rice, this potent synthetic auxin is frequently used as the sole growth regulator [46 and 47]. 2, 4-D causes DNA hyper-methylation that sustains exceedingly dynamic mitotic phase in cells and hence in a pro-embryonic stage [48 and 49]. Many researchers found high rate of callusing from various *indica* rice explants, after culturing on MS medium augmented with 2.0 mg/L concentration of 2, 4-D [50], while a lower or higher concentration of 2,4-D for callusing was also reported [1 and 51]. Several auxins *viz.* 2, 4-D, IAA (Indole-3-acetic acid) and NAA (1-Naphthaleneacetic acid) joined by a type of cytokinin at definite proportions were also used for callus induction in many rice cultivars [52].

6-Benzylaminopurine (BAP) is a synthetic cytokinin, which mainly endorse bud formation. BAP can promote cell differentiation when added in different media. Yan *et al.* (2010) [37] reported that BAP along with 2, 4-D increase the callusing rate. It is suggested that only 2, 4-D encourages callusing, inhibits roots and seedlings formation, whereas, BAP makes the callus to develop embryoid incessantly. Later on, higher callus

induction in *japonica* varieties was obtained using lower concentration of BAP [53]. The most reliable explanation is that BAP increases cell proliferation, which improves callus induction rate [54]. Pons *et al.* (2000) [55] found that BA is better than kinetin in indirect regeneration of rice independent of variety, whereas the choice of auxin is dependent on variety.

Thidiazuron [1-Phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea; TDZ] is one of numerous replaced ureas that have been explored for cytokinin functionality and have been discovered to stimulate multiple shooting in various plant species [56]. Apical meristem cells can multiply effectively with TDZ and be reprogrammed to proper developmental phase for shoot differentiation. [57]. TDZ at lower concentrations (10  $\mu\text{mol/L}$ ) is better than amino purine cytokinins (BAP) in inducing shoot regeneration of *indica* rice embryonic callus [58] and less prone to plant-degrading enzymes in comparison with other endogenous cytokinins [59]. Due to severe inhibition of shoot buds caused by greater TDZ concentrations (at 5 mg/L) being persistent in the tissues, regeneration, shoot proliferation, and development are decreased [3 and 60]. A relatively short time of TDZ exposure also stimulates plant regeneration [61]. Additionally, TDZ is active in the reprogramming and expression of competent cells, required for their differentiation and development. It was discovered that TDZ escalate synthesis, reduce catabolism and transform storage forms into physiologically dynamic cytokinins, which directed to an accretion of endogenous cytokinins. [62].

A combination of auxins, BAP, TDZ and other cytokinins are also reported to be operative in numerous shoot formation during regeneration of many plant species [63], which is frequently the expression of interfaces between physiological states of the explants [64]. Moreover, both synergistic and antagonistic properties among plant growth hormones are also testified in causing *in vitro* shoot proliferation [3]. It has been studied that different hormonal metabolisms run in a combined fashion with mutual functionally interacting points existing between them [65 and 66].

- 4. Media supplements:** Supplementation of *in vitro* culture media with amino acids has been testified to boost somatic embryogenesis. L-proline, L-glutamine, L-tryptophan, asparagine, glycine and casein hydrolysate (CH) are frequently used amino acid supplements for callusing and regeneration in *indica* rice [67 and 68]. L-Tryptophan is a precursor of the IAA, which is a vital auxin for cereals somatic embryogenesis [69]. CH acts as an special amino acid by facilitating callus induction in several rice genotypes [70]. Additionally, amino acids serve as a supply of reduced nitrogen, which plant cells can easily metabolize and use to speed up cell growth. Disparity in reactions of organic nitrogen foundations point to the necessity of precise amino acids for precise *in vitro* morphogenesis occasions. Hence, supplementary amino acids have the potential to enhance the roles of suitable nitrogen sources [71].
- 5. Gelling agent:** There are diverse gelling agents available that are used in making the culture medium solid and to influence plant development [72]. Agar, a complex polysaccharide obtained from algae is the commonly used gelling agent [73]. Agar concentration in the *in vitro* culture medium regulates its humidity, which influences the callus induction and embryogenic callus formation. However, the agar concentration at each stage of *in vitro* culture depends on genotype. Lower agar concentration facilitates the agility and absorption of nutrients present in the culture medium, stimulating the

callogenesis [74]. Agar also alters the availability of soluble materials through chemical reactions [75]. An alternative to agar is gelrite or phytigel, a complex extra-cellular polysaccharide formed by *Pseudomonas elodea*. It comprises less impurities and free minerals than agar. Gelrite also maintains stable pH in media unlike agar, where the pH frequently decreases as the culture ages [76]. Increased gel strength is linked to decreased water accessibility from the medium to the explants, irrespective of the solidifying agent type employed in culture medium [77]. This property is widely used for cell differentiation. Partial desiccation or the reduction of moisture content of callus and the treatment interval increases regeneration frequency in rice. Higher agarose content (more than 0.8% w/v) for medium solidification to increase the shoot formation frequency in *indica* rice calli even without any growth regulator treatment [14].

Therefore, it can be concluded that there are multiple factors work together in cell differentiation and dedifferentiation that are harnessed to produce the most suitable *indica* rice indirect regeneration system. The progress in last decade regarding this aspect is mentioned in **Table 1**.

**Table 1: Indica Rice Callus Induction and Indirect Regeneration from Mature Seeds: Progress in Last Decade**

Variety	Callus induction	Regeneration	Reference
White Ponni	MS + 2 mg/L 2, 4-D + 0.5 mg/L kinetin + 30 g/L sucrose	2.0 mg/L BAP + 0.5 mg/L NAA + 1.0 mg/L kinetin	Aananthi <i>et al.</i> , 2010 [78]
RD6 and RD15	NB + 30 g/L sucrose + 0.3 mg/L kinetin + 2 mg/L 2,4-D + 8 g/L agar	NB + 30 g/L sucrose, 1.0 mg/L IAA, 2 mg/L BA, 2 mg/L kinetin + 5 g/L phytigel	Darachai <i>et al.</i> , 2010 [79]
GNY-53, Basmati-370 and JP-5	3 mg/L 2, 4-D for GNY-53 and JP-5 1.0 mg/L 2,4-D for Basmati-370, MS medium better than N6 medium	1:2 mg/L combination of NAA and BAP for GNY-53 and JP-5. 1:4 mg/L for Basmati-370	Hussain <i>et al.</i> , 2010 [80]
MR 219	N6 + 2.5 mg/L 2,4-D + 0.2 mg/L kinetin+ 2.5 mg/L L-proline + 300 mg/L CH + 20 mg/L-glutamine + 30 g/L sucrose	MS + 3 mg/L BAP + 1.0 mg/L NAA + 2.5 mg/L-proline + 300 mg/L CH + 3% maltose	Sivakumar <i>et al.</i> , 2010 [81]
PAU 201 and PR 116	Semisolid MS + 2.5 mg/L 2,4-D + 0.5 mg/L kinetin + 560 mg/L proline + 30 g/L sucrose + 8 g/L agar	MS + 2.0 mg/L BAP + 0.5 mg/L kinetin + 0.5 mg/L NAA + 30 g/L sucrose + 8 g/L agar	Wani <i>et al.</i> , 2010 [82]
Kusan and Siam	2 mg/L 2, 4-D + 10 g/L sorbitol + MSB5 + 30 g/L sucrose + 0.4% gelrite	0.5 mg/L NAA + 2 mg/L kinetin + 2 mg/L BAP+ 20 g/L sorbitol	Shahsavari, 2011 [83]
Govind, Jaya, Pusa Basmati-1	12 µM 2,4-D for Govind in callusing	1.1 µM BAP or hormone free MS	Verma <i>et al.</i> , 2011 [84]
PAU 201	MS + 2.5 mg/L 2,4-D + 0.5 mg/L kinetin + 560 mg/L proline + 30 g/L sucrose	MS + 2.0 mg/L BAP + 0.5 mg/L NAA + 0.5 mg/L kinetin	Wani <i>et al.</i> , 2011 [85]
MR 219,	MS + 1.0 mg/L 2,4-D + 10	MS + 9 mg/L agar + 30 g/L	Zuraida <i>et al.</i> ,

MR 232	mg/L NAA + 30 g/L sucrose + 0.3% gelrite	sucrose + 10 mg/L ABA	2011 [86]
BRRRI dhan 28, 29, 47, 7	MS + 500 mg/L L-proline + 2.0 mg/L 2, 4-D + 0.8 mg/L BAP	MS + 6.0 mg/L kinetin + 0.5 mg/L NAA	Alam <i>et al.</i> , 2012 [87]
Sarawak rice var. Biris	2.0 mg/L 2, 4-D	0.5 mg/L NAA + 1.0 mg/L kinetin	Libin <i>et al.</i> , 2012 [88]
Kra Dang Ngah	MS + 2 mg/L 2,4-D + 1.0 mg/L NAA + 1.0 mg/L 6-BA + 0.5 mg/L kinetin callus proliferation in MS + 1.0 mg/L 2, 4-D + 0.5 mg/L IAA + 0.25 mg/L 6-BA + 0.25 mg/L kinetin	1.5 mg/L TDZ + 1.0 mg/L 2,4-D	Yinxia and Te-chato, 2012 [89]
Pakistani Basmati rice	5.0 mg/L 2,4-D for callogenesis and 1.0 mg/L 2,4-D for regeneration	maltose 30 g/L, kinetin 3 mg/L, NAA 1.0 mg/L 30 g/L maltose, 2.6 g/L phytigel	Joyia and Khan, 2013 [90]
IR36	11.31 $\mu$ M 2, 4-D + 0.3 $\mu$ M kinetin	MS + 13.28 $\mu$ M BA + 8.06 $\mu$ M NAA	Krishnan <i>et al.</i> , 2013 [91]
Kalijira, Chinigura	MS + 2 mg/L 2,4-D	MS + 0.5 mg/L BAP + 0.1 mg/L IBA	Mannan <i>et al.</i> , 2013 [92]
landrace Hom Kra Dang Ngah	MS + 2 mg/L 2,4-D + 1.0 mg/L NAA + 1.0 mg/L 6-BA + 0.5 mg/L kinetin + 3% sucrose + 1.0 g/L CH	ARDA + 0.5 mg/L NAA + 1.0 mg/L 6-BA + 2 mg/L kinetin + 82 mM sorbitol MS + 1.0 g/L CH + 0.3% phytigel	Yinxia and Te-chato, 2013 [93]
BRRRI dhan 52, FR13A	MS + 3 mg/L 2, 4-D	MS + 2 mg/L kinetin + 2 mg/L BA + 1.0 mg/L NAA	Bhuiyan <i>et al.</i> , 2014 [94]
FEDEARROZ 2000	MS basal medium + 30 g/L sucrose + 0.5 g/L proline + 0.5 g/L CH + 2.5 g/L gelrite, pH 5.8	1 mg/L NAA + 2 mg/L kinetin	Barbosa Cepeda and Chaparro-Giraldo, 2014 [95]
BRRRI dhan 28, 29, 30, 34, 56, 57	2 mg/L 2,4-D + 0.5 mg/L NAA	2 mg/L BAP + 1.0 mg/L NAA + 1.5 mg/L kinetin	Islam <i>et al.</i> , 2014 [96]
Aromatic rice	2 mg/L 2, 4-D + 0.5 mg/L NAA	0.5 mg/L BAP + 0.1 mg/L IBA	Roly <i>et al.</i> , 2014 [97]
IR64	MS + 3 mg/L 2, 4-D + 400 mg/L proline + 200 mg/L CH	2.5 mg/L BAP + 400 mg/L proline + 200 mg/L CH	Toppo <i>et al.</i> , 2014 [98]
Nemat and Dom siah	MS + 2 mg/L 2,4-D + 30 g/L sucrose + 7 g/L agar	3 mg/L AgNO <sub>3</sub> + 9 g/L agar + 20 g/L PEG for somatic embryogenesis 9-11 g/L agar + 5 mg/L AgNO <sub>3</sub> for regeneration	Ghobeishavi <i>et al.</i> , 2015 [99]
BRRRI dhan29, 28	MS + 2.5 mg/L 2,4-D + 0.5 mg/L 6-BAP	MS + 0.6 mg/L NAA + 6 mg/L kinetin	Hossain <i>et al.</i> , 2015 [100]
BRRRI dhan56	MS + 2.5 mg/L 2, 4-D + 0.5 mg/L NAA + 0.8% agar	BAP 2.0 mg/L + 1.0 mg/L NAA + 1.5 mg/L kinetin	Islam <i>et al.</i> , 2015 [101]
Pusa Sugandha,	1.0 mg/L 2,4-D	0.5 mg/L 2,4-D + 1.0 mg/L kinetin + 0.5 mg/L BAP	Mahajan and Sharma, 2015

Pusa1 and Pusa 1121			[102]
IR 64	MS + vitamin B5 + 30 g/L sucrose + 0.3 g/L CH + 2.5 mg/L 2,4-D + 0.1 mg/L BAP + 0.65 g/L proline + 4 g/L phytigel, pH 5.8.	MS + vitamin B5 + 30 g/L maltose + 2.7 mg/L BAP + 1.2 mg/L kinetin + 0.5 mg/L NAA	Tran and Mishra, 2015 [103]
AC39020	LS + 2.5 mg/L 2, 4-D + 500 mg/L glutamine	MS + 4 mg/L BAP + 0.5 mg/L NAA	Vennapusa <i>et al.</i> , 2015 [104]
Swarna	2.0 mg/L 2,4, D + 0.5 mg/L kinetin+ MSB5 + 0.1 g/L myo-inositol + 0.5 g/L CH + 0.6 g/L L-proline + 30 g/L maltose + 4 g/L phytigel	2.0 mg/L kinetin + 0.5 mg/L NAA + MSB5+ 0.1 g/L myo-inositol + 2.0 g/L CH + 30 g/L sucrose + 5 g/L phytigel	Juturu <i>et al.</i> , 2016 [105]
Thai Rice Variety: Nam Roo	NB + 1 mg/L 2, 4-D + 0.5 mg/L NAA + 1.0 g/L L-proline + 30 g/L sucrose + 2.6 g/L phytigel	2 mg/L BAP + 5.2 g/L phytigel	Poeaim <i>et al.</i> , 2016 [106]
Jow Haw rice	NB + 3 mg/L 2,4-D	MS + 2 mg/L BAP + 5.2 g/L phytigel	Poraha <i>et al.</i> , 2016 [107]
P-44, PR-115 and 116, PAV-16 and 201, Sarsu 52,	3.0 mg/L 2,4-D	5.0 mg/L BAP	Sankepally and Singh, 2016 [108]
Balinese red rice	MS + 0.75 mg/L 2,4-D	MS + 5 mg/L BAP + 0.2 mg/L TDZ	Artadana <i>et al.</i> , 2017 [109]
BRR1 Dhan 28, 29	4 mg/L 2, 4-D	MS + 1.5 mg/L BA + 0.5 mg/L NAA	Chakraborty <i>et al.</i> , 2017 [110]
Malaysian rice	MS + 3 mg/L 2,4-D	MS + 3 mg/L BAP + 2 mg/L kinetin + 0.5 mg/L NAA + 30 g/L maltose + 4 g/L gelrite	Mostafiz <i>et al.</i> , 2018 [111]
Malaysian rice MR220, MR220-CL2, MR232, Bario	MS + 3 mg/L 2,4-D + 30 g/L maltose	MS + 2 mg/L BAP + 2 mg/L kinetin + 0.5 mg/L NAA	Binte Mostafiz and Wagiran, 2018 [47]
Pakaumpuel Thai rice	MS + 2 mg/L 2,4-D	1 mg/L NAA + 3 mg/L BAP	Trunjaruen <i>et al.</i> , 2018 [112]
Binadhan-5, 6, 48, 58, IR-64	MS + Sucrose + 2 mg/L 2,4-D	MS salts + 30 g/L maltose + 2 mg/L kinetin + 0.2 mg/L NAA + 8 g/L agar	Khan <i>et al.</i> , 2019 [113]
Malaysian recalcitrant <i>indica</i> rice cv. MR219	Gamborg's B5 + 10 g/L maltose + 10 mg/L NAA + 1.0 mg/L 2,4-D and MS + 2 mg/L 2,4-D + 0.5 mg/L kinetin + 100 mg/L lignosulfonate for proliferation	MS + 30 g/L sucrose + 3 mg/L kinetin + 100 mg/L CaLS	Lee <i>et al.</i> , 2019 [114]
Aromatic <i>indica</i> rice	3 mg/L 2,4-D + 30 g/L sucrose + 8 g/L agar	1.0 mg/L NAA + 2 mg/L BAP + 4 mg/L kinetin	Paul and Roychoudhury, 2019 [115]



Kavuni	NB + 2 mg/L 2,4-D + 1.0 mg/L NAA + 1.0 mg/L 6-BA + 2% sucrose + 1% glucose	3 mg/L 6-BA + 0.5 mg/L kinetin + 3% maltose + 0.3 g/L glutamine	Rakshana <i>et al.</i> , 2019 [116]
Lakhai	MS + 3 mg/L NAA + 1.0 mg/L BA	0.5 mg/L NAA + 3.0 mg/L BA	Hasan <i>et al.</i> , 2020 [117]
CO 51	NB + NAA 1.0 mg/L + 1.0 mg/L 6-BA + 2.5 mg/L 2,4-D	3 mg/L 6-BA + 1.0 mg/L NAA	Shweta <i>et al.</i> , 2020 [118]
ASD16, IR64, and ADT43	N6 + 30 g/L sucrose + 2 mg/L 2,4-D	MS + 2.0 mg/L BAP + 1.0 mg/L NAA + 30 g/L sucrose + 1.0 mM putrescine	Sundararajan <i>et al.</i> , 2021 [119]
BRRRI Dhan 58	MS + 2.5 mg/L 2, 4-D	MS + 3 mg/L BAP + 0.5 mg/L NAA + 1.0 mg/L kinetin	Banu <i>et al.</i> , 2021 [120]
Fatmawati	NB5 + 3 mg/L 2,4-D + 8 g/L agar + 0.1 g/L myo-inositol + 30 g/L sucrose + 100 mL/L fresh coconut water + 0.5 g/L L-proline + 0.5 g/L L-glutamine + 30 g/L maltose	NB5 + 12 g/L agar + 100 mL/L coconut water + 0.1 g/L myo-inositol + 0.5 mg/L NAA + 0.5 mg/L IAA + 0.5 g/L L-proline + 0.5 g/L L-glutamine + 3 mg/L kinetin + 0.8 g/L CH, 3 mg/L BA + 30 g/L maltose	Carsono <i>et al.</i> , 2021 [121]
Indica rice from Bangladesh	N6 + 2.5-3.0 mg/L 2,4-D	2.5 mg/L BA + 1.0 mg/L NAA + 3 mg/L 6-BA + 1.5 mg/L NAA	Hasan <i>et al.</i> , 2021 [122]
Chittimuthyalu	MS + 2.5 mg/L 2, 4-D + 0.5 mg/L kinetin	MS + 0.5 mg/L NAA + 2 mg/L BAP	Tripathy, 2021a [123]
Khandagiri, Sahbhagidhan, Mandakini	MS + 2.5 mg/L 2,4-D + 0.5 mg/L kinetin + 3% sucrose + 0.3% agar + 0.2% phytagel + 500 mg/L CH + 150 mg/L proline	MS + 2.5% sucrose + 0.3% agar + 0.2% phytagel + 500 mg/L CH + 500 mg/L adenine sulphate + 150 mg/L proline + 2.0 mg/L 6-BAP + 0.5 mg/L NAA	Tripathy, 2021b [124]

**MS**= Murashige and Skoog medium, **NB**= Macro elements of N6 medium (Chen *et al.*, 1998) + microelements and vitamins of B5 medium (Gamborg *et al.*, 1968), **BA**= Benzyladenine, **BAP**= Benzylaminopurine, **NAA**= 1-Naphthaleneacetic acid, **TDZ**= Thidiazuron, **IAA**= Indole-3-acetic acid, **ABA**= Abscisic acid, **IBA**= Indole-3-butyric acid, **CH**= Casein hydrolysate

The interaction of auxin and cytokinin at a specific ratio controls cellular differentiation during plant tissue culture. Their combination is used in embryogenic callus regeneration of some rice cultivars. In Table 1, the conditions employed for callusing and regeneration are mentioned, which include, basal medium, growth regulators, gelling agent, growth supplements, carbon source etc. Most of these compositions are similar or standard for every experiment, according to the respective growth stage. However, above all the other factors, the plant growth regulator combination, concentration and their type is mostly altered to obtain suitable regeneration for particular *indica* rice genotypes. Therefore, the major scope to establish and improve genotype dependent *indica* rice regeneration system lies within tweaking the plant growth regulators according to their amenability [125].

### III. CONCLUSION

*In vitro* regeneration occurs in two main ways including organogenesis and somatic embryogenesis. The formation of bipolar organisations from somatic cells (haploid or diploid) without any gamete fusion (not attached to mother calli vascular tissues) is known as somatic embryogenesis. Somatic embryogenesis is a special developmental route that only befalls in plants, and largely exploited in biotechnological interventions like clonal propagation, the creation of synthetic seeds, and genetic modification. Somatic embryogenesis is a useful tool to support crop species genetic improvement when combined with traditional breeding programmes and molecular biology techniques. There are numerous purposes for which indirect regeneration of rice is utilised. These are establishment of a protocol that is amenable for genetic transformation, studying the functions of growth hormones, media supplements, additives and unexplored materials that have the potential to optimize the process in different rice genotypes. The most prevalent regeneration mechanism in rice is somatic embryogenesis, which has been seen in caryopses, early inflorescences, immature or mature embryos, roots, the leaf bases of young seedlings, coleoptiles, cell suspension and protoplast. The most commonly used explant for indirect regeneration of rice *i.e.* through somatic embryogenesis is mature seed derived embryos. The detailed literature study revealed that there are few conditions available, which are standard in every study. These are: MS basal medium, common growth hormones like auxin (IAA, IBA, NAA, 2,4-D), cytokinin (BAP, kinetin, TDZ), supplements (proline, casein, ascorbic acid, glutamine, tryptophan), carbon source (sucrose, maltose), gelling agents (agar, agarose, phytigel/gelrite) etc. However, their concentrations, combinations often varies according to the genotype, subspecies, developmental stage etc. Therefore, while starting an indirect regeneration, these conditions are need to be optimized invariably. This article is a document of detailed progress on rice indirect regeneration done in last decade.

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