

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

Authors

M. Sutradhar

Department of Agricultural Biotechnology
Faculty of Agriculture
Bidhan Chandra Krishi Viswavidyalaya
Mohanpur, Nadia, West Bengal, India
monoj.gene.enggmr@gmail.com

N. Mandal

Department of Agricultural Biotechnology
Faculty of Agriculture
Bidhan Chandra Krishi Viswavidyalaya
Mohanpur, Nadia, West Bengal, India

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 a 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 μ M 2, 4-D + 0.3 μ M kinetin	MS + 13.28 μ M BA + 8.06 μ 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 ₃ + 9 g/L agar + 20 g/L PEG for somatic embryogenesis 9-11 g/L agar + 5 mg/L AgNO ₃ 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]
BRRDhan 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% phytigel + 500 mg/L CH + 150 mg/L proline	MS + 2.5% sucrose + 0.3% agar + 0.2% phytigel + 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.

REFERENCES

- [1] Z. Abd, Rahman, Z. A. Seman, A. N. Othman, M. B. Ab Ghaffar, S. Ab Razak, M. F. M. Yusof, K. H. Nasir, K. Ahmad, Y. L. Chow, and S. Subramaniam, "Efficient callus induction and plant regeneration of Malaysian *indica* rice MR219 using anther culture," *Biocatal. Agric. Biotechnol.*, vol. 31, pp. 101865, January 2021.
- [2] S. Dixit, U. M. Singh, A. K. Singh, S. Alam, C. Venkateshwarlu, V. V. Nachimuthu, S. Yadav, R. Abbai, R. Selvaraj, M. N. Devi, and P. J. Ramayya, "Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/tolerance in rice," *Rice*, vol. 13(1), pp. 1-15, December 2020.
- [3] M. Dey, S. Bakshi, G. Galiba, L. Sahoo, and S. K. Panda, "Development of a genotype independent and transformation amenable regeneration system from shoot apex in rice (*Oryzasativa* spp. *indica*) using TDZ," *3 Biotech*, vol. 2(3), pp. 233-240, September 2012.
- [4] R. T. Furbank, W. P. Quick, and X. R. Sirault, "Improving photosynthesis and yield potential in cereal crops by targeted genetic manipulation: prospects, progress and challenges," *Field Crops Res.*, vol. 182, pp. 19-29, October 2015.
- [5] T. P. Michael, "Plant genome size variation: bloating and purging DNA," *Brief. Funct. Genom.*, vol. 13(4), pp. 308-317, July 2014.
- [6] Y. Indoliya, P. Tiwari, A. S. Chauhan, R. Goel, M. Shri, S. K. Bag, and D. Chakrabarty, "Decoding regulatory landscape of somatic embryogenesis reveals differential regulatory networks between *japonica* and *indica* rice subspecies," *Sci. Rep.*, vol. 6(1), pp. 1-15, March 2016.

- [7] X. Xu, X. Liu, S. Ge, J. D. Jensen, F. Hu, X. Li, Y. Dong, R. N. Gutenkunst, L. Fang, L. Huang, J. Li, W. He, G. Zhang, X. Zheng, F. Zhang, Y. Li, C. Yu, K. Kristiansen, X. Zhang, J. Wang, M. Wright, S. McCouch, R. Nielsen, J. Wang, and W. Wang, "Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes," *Nat. Biotechnol.*, vol. 30, pp. 105–111, January 2012.
- [8] A. Ziemienowicz, "Agrobacterium-mediated plant transformation: factors, applications and recent advances," *Biocatal. Agric. Biotechnol.*, vol. 3(4), pp. 95-102, October 2014.
- [9] H. H. Hwang, M. Yu, and E. M. Lai, "Agrobacterium-mediated plant transformation: biology and applications," in *The Arabidopsis Book* vol. 15 American Society of Plant Biologists. Monona Drive Rockville, USA. pp. e0186, October 2017.
- [10] I. B. Holme, T. Wendt, and P. B. Holm, "Intragenesis and cisgenesis as alternatives to transgenic crop development," *Plant Biotechnol. J.*, vol. 11(4), pp. 395-407, May 2013.
- [11] L. Van Hove, and F. Gillund, "Is it only the regulatory status? Broadening the debate on cisgenic plants," *Environ. Sci. Eur.*, vol. 29(1), pp. 1-11, December 2017.
- [12] S. Jang, S. C. Choi, H. Y. Li, G. An, and E. Schmelzer, "Functional characterization of *Phalaenopsisaphrodite* flowering genes PaFT1 and PaFD," *PloS one*, vol. 10(8), pp. 0134987, August 2015.
- [13] B. Lacroix, and V. Citovsky, "Pathways of DNA transfer to plants from *Agrobacteriumtumefaciens* and related bacterial species," *Annu. Rev. Phytopathol.*, vol. 57, pp. 231-251, August 2019.
- [14] K. K. Sahoo, A. K. Tripathi, A. Pareek, S. K. Sopory, and S. L. Singla-Pareek, "An improved protocol for efficient transformation and regeneration of diverse *indica* rice cultivars," *Plant methods*, vol. 7(1), pp. 1-11, December 2011.
- [15] B. D. Pawar, A. S. Jadhav, A. A. Kale, V. P. Chimote, and S. V. Pawar, "Effect of explants, bacterial cell density and overgrowth-control antibiotics on transformation efficiency in tomato (*Solanumlycopersicum* L.)," *J. Appl. Hortic.*, vol. 15(2), pp.95-99, May 2013.
- [16] L. Yang, W. Hu, Y. Xie, Y. Li, and Z. Deng, "Factors affecting *Agrobacterium*-mediated transformation efficiency of kumquat seedling internodal stem segments," *Sci. Hortic.*, vol. 209, pp. 105-112, September 2016.
- [17] L. Satish, S. A. Ceasar, and M. Ramesh, "Improved *Agrobacterium*-mediated transformation and direct plant regeneration in four cultivars of finger millet (*Eleusinecoracana* L. Gaertn.)," *Plant Cell Tissue Organ Cult.*, vol. 131(3), pp. 547-565, September 2017.
- [18] J. Yu, W. Liu, J. Liu, P. Qin, and L. Xu, "Auxin control of root organogenesis from callus in tissue culture," *Front. Plant Sci.*, vol. 8, pp. 01385, August 2017.
- [19] S. S. Bidabadi, and S. M. Jain, "Cellular, molecular, and physiological aspects of *in vitro* plant regeneration," *Plants*, vol. 9(6), pp. 702, June 2020.
- [20] G. C. Phillips, and M. Garda, "Plant tissue culture media and practices: an overview," *In Vitro Cell. Dev. Biol. Plant*, vol. 55(3), pp. 242-257, June 2019.
- [21] S. Sundararajan, B. Sivaraman, V. Rajendran, and S. Ramalingam, "Tissue culture and *Agrobacterium*-mediated genetic transformation studies in four commercially important *indica* rice cultivars," *J. Crop Sci. Biotechnol.*, vol. 20(3), pp. 175-183, September 2017.
- [22] P. Puhan, R. K. Nagireddy, L. R. Vemireddy, and E. A. Siddiq, "Effect of *NiR* gene on *in vitro* regeneration protocol of *indica*, *japonica*, aromatic and wild rice varieties," *ORYZA-An Int. J. Rice.*, vol. 55(4), pp. 500-510, December 2019.
- [23] M. Molina-Risco, O. Ibarra, M. Faion-Molina, B. Kim, E. M. Septiningsih, and M. J. Thomson, "Optimizing *Agrobacterium*-mediated transformation and CRISPR-Cas9 gene editing in the tropical *japonica* rice variety presidio," *Int. J. Mol. Sci.*, vol. 22(20), pp. 10909, October 2021.
- [24] S. Mohammed, A. Abd Samad, and Z. Rahmat, "Agrobacterium-mediated transformation of rice: constraints and possible solutions," *Rice Sci.*, vol. 26(3), pp. 133-146, May 2019.
- [25] M. Dey, S. K. Panda, and L. Sahoo, "Establishment of an efficient regeneration system amenable to *Agrobacterium*-mediated transformation of two elite *indica* rice varieties of northeast India," *Int. J. Appl. Sci. Biotechnol.*, vol. 3(4), pp. 680-686, December 2015.

- [26] M. Solanki, A. Sinha, and L. I. Shukla, "Optimization of *in vitro* culture media for improvement in yield of Navara ancient Indian medicinal rice," 3 Biotech, vol. 9(7), pp. 1-13, July 2019.
- [27] A. Sarkar, I. Srinivasan, and S. Roy Barman, "Optimisation of a rapid and efficient transformation protocol for fungal blast-susceptible *indica* rice cultivars HR-12 and CO-39," Plant Biotechnol., vol. 38(4), pp. 433-441, December 2021.
- [28] B. Ijaz, C. Sudiro, M. Z. Hyder, S. I. Malik, S. Farrakh, F. L. Schiavo, and T. Yasmin, "Histomorphological analysis of rice callus cultures reveals differential regeneration response with varying media combinations," *In Vitro Cell. Dev. Biol. Plant*, vol. 55(5), pp. 569-580, October 2019.
- [29] H. Hisano, T. Matsuura, I. C. Mori, M. Yamane, and K. Sato, "Endogenous hormone levels affect the regeneration ability of callus derived from different organs in barley," Plant Physiol. Biochem., vol. 99, pp. 66-72, February 2016.
- [30] Y. H. Su, L. P. Tang, X. Y. Zhao, and X. S. Zhang, "Plant cell totipotency: Insights into cellular reprogramming," J. Integr. Plant Biol., vol. 63(1), pp. 228-243, January 2021.
- [31] P. Kumar, and D. K. Srivastava, "Biotechnological applications in *in vitro* plant regeneration studies of broccoli (*Brassicaoleracea* L. var. Italica), an important vegetable crop," Biotechnol. Lett., vol. 38(4), pp. 561-571, April 2016.
- [32] R. Mishra, and G. J. N. Rao, "*In vitro* androgenesis in rice: advantages, constraints and future prospects," Rice Sci., vol. 23(2), pp. 57-68, March 2016.
- [33] H. Ghareeb, U. Aly, A. El Kazzaz, and M. Hanafy, "Optimization of rice regeneration system from mature seeds of five Egyptian rice cultivars," Afr. J. Plant Sci. Biotechnol., vol. 3(1), pp. 63-66, October 2009.
- [34] J. Ali, K. L. C. Nicolas, S. Akther, A. Torabi, A. A. Ebadi, C. M. Marfori Nazarea, and A. Mahender, "Improved anther culture media for enhanced callus formation and plant regeneration in rice (*Oryzasativa* L.)," Plants, vol. 10(5), pp. 839, April 2021.
- [35] A. Maharani, W. I. D. Fanata, F. N. Laeli, K. M. Kim, and T. Handoyo, "Callus induction and regeneration from anther cultures of Indonesian *indica* black rice cultivar," J. Crop Sci. Biotechnol., vol. 23(1), pp. 21-28, January 2020.
- [36] J. Hou, H. Chen, Y. Fang, Y. Zhu, B. Han, C. Sun, and Y. Fu, "An *Agrobacterium*-mediated non-antibiotic selection-based transformation system for rice (*Oryzasativa* ssp. *indica*) cultivar 93-11 successfully produces TAC1-silenced transgenic plants," *In Vitro Cell. Dev. Biol. Plant*, vol. 57(5), pp.786-795, October 2021.
- [37] L. N. Yan, L. I. Xia, and W. U. Dan, "The comparison in tissue culture ability of mature embryo in different cultivars of rice," Agric. Sci. China, vol. 9(6), pp. 840-846, June 2010.
- [38] A. Pazuki, J. Asghari, M. M. Sohani, M. Pessarakli, and F. Aflaki, "Effects of some organic nitrogen sources and antibiotics on callus growth of *indica* rice cultivars," J. Plant Nutr., vol. 38(8), pp. 1231-1240, July 2015.
- [39] U. Yaqoob, T. Kaul, and I. A. Nawchoo, "Development of an efficient protocol for *Agrobacterium* mediated transformation of some recalcitrant *indica* rice varieties," Indian J. Plant Physiol., vol. 22(3), pp. 346-353, September 2017.
- [40] E. J. Oliveira, A. D. Koehler, D. I. Rocha, L. M. Vieira, M. V. M. Pinheiro, E. M. de Matos, A. C. F. da Cruz, T. C. R. da Silva, F. A. O. Tanaka, F. T. S. Nogueira, and W. C. Otoni, "Morpho-histological, histochemical, and molecular evidences related to cellular reprogramming during somatic embryogenesis of the model grass *Brachypodiumdistachyon*," Protoplasma, vol. 254(5), pp. 2017-2034, March 2017.
- [41] H. Chauhan, and P. Khurana, "Wheat genetic transformation using mature embryos as explants," Wheat Biotechnology, (Eds. Bhalla, P. L. and Singh, M. B.). Humana Press, New York, NY. pp. 153-167, September 2017.
- [42] L. H. Abdul-Qadir, (2016). "Callus induction and somatic embryogenesis of rice (*Oryzasativa* L.) improvement with the addition of coconut water," J. Biol. Agric. Healthc., vol. 6(2), pp.12-17.

- [43] N. N. Kruglova, G. E. Titova, O. A. Seldimirova, and A. E. Zinatullina, "Cytophysiological features of the cereal-based experimental system embryo *in vivo*–callus *in vitro*," Russ. J. Dev. Biol., vol. 52(4), pp. 199-214, July 2021.
- [44] N. Kral, A. Hanna Ougolnikova, and G. Sena, "Externally imposed electric field enhances plant root tip regeneration," Regeneration, vol. 3(3), pp. 156-167, June 2016.
- [45] A. Fehér, "Somatic embryogenesis—stress-induced remodelling of plant cell fate," Biochim. Biophys. Acta - Gene Regul. Mech., vol. 1849(4), pp. 385-402, April 2015.
- [46] K. Ozawa, H. Kawahigashi, T. Kayano, and Y. Ohkawa, "Enhancement of regeneration of rice (*Oryzasativa* L.) from calli involved in the integration of gene involved in the regeneration ability of the callus," Plant Sci., vol. 16(5), pp. 395-402, August, 2003.
- [47] S. Binte Mostafiz, and A. Wagiran, "Efficient callus induction and regeneration in selected *indica* rice," Agronom., vol. 8(5), pp. 77, April 2018.
- [48] A. Meneses, D. Flores, M. Muñoz, G. Arrieta, A. M. Espinoza, "Effect of 2,4-D, hydric stress and light on *indica* rice (*Oryzasativa*) somatic embryogenesis," Rev. Biol. Trop., vol. 53(3-4), pp. 361-368, September 2005.
- [49] W. Wang, Q. Qin, F. Sun, Y. Wang, D. Xu, Z. Li, and B. Fu, "Genome-wide differences in DNA methylation changes in two contrasting rice genotypes in response to drought conditions," Front. Plant Sci., vol. 7, pp. 1675, November 2016.
- [50] G. Upadhyaya, M. Sen, and A. Roy, "In vitro callus induction and plant regeneration of rice (*Oryzasativa* L.) var. Sita, Rupali and Swarna Masuri," Asian J. Plant Sci. Res., vol. 5(5), pp. 24-27, 2015.
- [51] B. Liu, H. Wu, S. Yang, E. Wu, P. Yang, and X. Gao, "Efficient callus induction and regeneration in proso millet," Agron. J., vol. 113(5), pp. 4003-4012, September 2021.
- [52] G. Chaâbani, J. Tabart, C. Kevers, J. Dommès, M. I. Khan, S. Zaoui, L. Chebchoub, M. Lachaâl, and N. Karray Bouraoui, "Effects of 2, 4-dichlorophenoxyacetic acid combined to 6-benzylaminopurine on callus induction, total phenolic and ascorbic acid production, and antioxidant activities in leaf tissue cultures of *Crataegus azarolus* L. var. aronia," Acta Physiol. Plant., vol. 37(2), pp. 1-9, February 2015.
- [53] M. Feng, J. Cang, J. Wang, J. Sun, J. Yu, Q. Xu, D. Zhang, N. Yang, Q. Lu, and Y. Lv, "Regeneration and *Agrobacterium*-mediated transformation of *japonica* rice varieties developed for a cold region," Czech J. Genet. Plant Breed., vol. 54(4), pp. 161-167, November 2018.
- [54] Huang, M., Fu, L., Sun, X., Di, R. and Zhang, J. "Rapid and highly efficient callus induction and plant regeneration in the starch-rich duckweed strains of *Landoltia punctata*," Acta Physiol. Plant., vol. 38(5), pp. 1-13, May 2016.
- [55] M. J. Pons, V. Marfà, E. Melé, and J. Messegue, "Regeneration and genetic transformation of Spanish rice cultivars using mature embryos," Euphytica, vol. 114(2), pp. 117-122, July 2000.
- [56] S. R. Pai, and N. S. Desai, "Effect of TDZ on various plant cultures," in Thidiazuron: From urea derivative to plant growth regulator, (Eds. Ahmad, N. and Faisal, M.). Springer Singapore. pp. 439-454, March 2018.
- [57] E. T. Dinani, M. R. Shukla, C. E. Turi, J. A. Sullivan, and P. K. Saxena, "Thidiazuron: modulator of morphogenesis *in vitro*," in Thidiazuron: from urea derivative to plant growth regulator, (Eds. Ahmad, N. and Faisal, M.). Springer Singapore. pp. 1-36, March 2018.
- [58] A. Gairi, and A. Rashid, "TDZ-induced somatic embryogenesis in non-responsive caryopses of rice using a short treatment with 2, 4-D," Plant Cell Tissue Organ Cult., vol. 76, pp. 29-33, January 2004.
- [59] S. Rajput, and V. Agrawal, "Micropropagation of *Atropa acuminata* Royle ex Lindl. (a critically endangered medicinal herb) through root callus and evaluation of genetic fidelity, enzymatic and non-enzymatic antioxidant activity of regenerants," Acta Physiol. Plant., vol. 42(11), pp. 1-17, November 2020.
- [60] A. V. Deepa, M. Anju, and T. Dennis Thomas, "The applications of TDZ in medicinal plant tissue culture," in Thidiazuron: from urea derivative to plant growth regulator, (Eds. Ahmad, N. and Faisal, M.). Springer Singapore. pp. 297-316, March 2018.

- [61] Y. H. Dewir, Y. Naidoo, and J. A. Teixeira da Silva, "Thidiazuron-induced abnormalities in plant tissue cultures," *Plant Cell Rep.*, vol. 37(11), pp. 1451-1470, November 2018.
- [62] P. Bhattacharya, V. Kumar, and J. Van Staden, "*In vitro* encapsulation based short term storage and assessment of genetic homogeneity in regenerated *Anselliaafricana* (Leopard orchid) using gene targeted molecular markers," *Plant Cell Tissue Organ Cult.*, vol. 133(2), pp. 299-310, May 2018.
- [63] S. Sadhu, P. Jogam, R. K. Thampu, S. Abbagani, S. Penna, and V. Peddaboina, "High efficiency plant regeneration and genetic fidelity of regenerants by SCoT and ISSR markers in chickpea (*Cicerarietinum* L.)," *Plant Cell Tissue Organ Cult.*, vol. 141(3), pp. 465-477, June 2020.
- [64] D. Kulus, "Influence of growth regulators on the development, quality, and physiological state of *in vitro* propagated *Lamprocapnos spectabilis* (L.) Fukuhara," *In Vitro Cell. Dev. Biol. Plant*, vol. 56(4), pp. 447-457, August 2020.
- [65] T. Gaspar, B. Bisbis, C. Kevers, T. Franck, J. Dommès, M. Crevecouer, C. Penel, and H. Greppin, "Integrating phytohormone metabolism and action with primary biochemical pathways. II. Interrelationships between disturbed nitrogen and carbon metabolism and changes in hormonal concentrations and sensitivities in tissue cultures," *Integrated plant systems*, (Eds. Greppin, H., Penel, C., Broughton, W. and Strasser, R.). University of Geneve, Geneva, pp. 139-225, 2000.
- [66] O. Berkowitz, I. De Clercq, F. Van Breusegem, and J. Whelan, "Interaction between hormonal and mitochondrial signalling during growth, development and in plant defence responses," *Plant Cell Environ.*, vol. 39(5), pp. 1127-1139, May 2016.
- [67] M. A. Daniel, R. H. A. David, S. A. Caesar, M. Ramakrishnan, V. Duraipandiyan, S. Ignacimuthu, and N. A. Al Dhabhi, "Effect of l-glutamine and casein hydrolysate in the development of somatic embryos from cotyledonary leaf explants in okra (*Abelmoschuseculentus* L. Monech)," *S. Afr. J. Bot.*, vol. 114, pp. 223-231, January 2018.
- [68] J. J. Rybczynski, and A. I. Wojcik, "The effect of L-glutamine on the genetic transformation of embryogenic cell suspensions of gentian species (*Gentianalutea* L., *Gentianacruciata* L., and *Gentianakurroo* Royle) using *Agrobacteriumtumefaciens*," *BioTechnologia. J. Biotechnol. Comput. Biol. Bionanotechnol.*, vol. 100(1), pp. 5-18, March 2019.
- [69] F. I. Ahmad, A. Wagiran, A. Abd Samad, Z. Rahmat, and M. R. Sarmidi, "Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryzasativa* cv. Panderas)," *Saudi J. Biol. Sci.*, vol. 23(1), pp. S69-S77, January 2016.
- [70] A. M. Amer, G. M. Mohamed, M. H. Hussein, M. Z. Sedik, and U. I. Aly, "Effect of some of the natural organic sources on rice tissue culture," *Egypt. Pharm. J.*, vol. 16(3), pp. 152-156, September 2017.
- [71] R. Abiri, M. Maziah, N. A. Shaharuddin, Z. N. B. Yusof, N. Atabaki, M. M. Hanafi, M. Sahebi, P. Azizi, N. Kalthori, and A. Valdiani, "Enhancing somatic embryogenesis of Malaysian rice cultivar MR219 using adjuvant materials in a high efficiency protocol," *Int. J. Environ. Sci. Technol.*, vol. 14(5), pp. 1091-1108, May 2017.
- [72] L. A. Nery, D. S. Batista, D. I. Rocha, S. H. S. Felipe, M. D. C. Queiroz, P. O. Silva, M. C. Ventrella, and W. C. Otoni, "Leaf development and anatomy of *in vitro* grown *Polygalapaniculata* L. are affected by light quality, gelling agents, and sucrose," *Vegetos*, vol. 34(1), pp. 19-28, March 2021.
- [73] W. K. Lee, Y. Y. Lim, A. T. C. Leow, P. Namasivayam, J. O. Abdullah, and C. L. Ho, "Factors affecting yield and gelling properties of agar," *J. Appl. Phycol.*, vol. 29(3), pp. 1527-1540, June 2017.
- [74] M. Pérez Bernal, C. Hernández, M. T. Barceló, M. Delgado, and R. Armas, "Quantitative transient GUS expression in J-104 rice calli through manipulation of *in vitro* culture conditions," *Rev. Colomb. Biotecnol.*, vol. 11(2), pp. 75-84, June 2009.

- [75] M. Abdoli, A. Moieni, and H. Dehghani, "Effects of cultivar and agar concentration on *in vitro* shoot organogenesis and hyperhydricity in *Helianthus annuus* L.," Pak. J. Bot., vol. 39, pp. 31-35, February 2007.
- [76] G. M. Mohamed, A. M. Amer, N. H. Osman, M. Z. Sedikc, and M. H. Hussein, "Effects of different gelling agents on the different stages of rice regeneration in two rice cultivars," Saudi J. Biol. Sci., vol. 28(10), pp. 5738-5744, October 2021.
- [77] K. Klimaszewska, M. Bernier-Cardou, D. R. Cyr, and B. C. S. Sutton, "Influence of gelling agents on culture medium gel strength, water availability, tissue water potential, and maturation response in embryogenic cultures of *Pinus strobus* L.," *In Vitro Cell. Dev. Biol. Plant*, vol. 36(4), pp. 279-286, July 2000.
- [78] N. Aananthi, C. R. Anandakumar, R. Ushakumari, and P. Shanthi, "Regeneration study of some *indica* rice cultivars followed by *Agrobacterium*-mediated transformation of highly regenerable cultivar, Pusa Basmati 1," *Electron. J. Plant Breed.*, vol. 1(4), pp. 1249-1256, July 2010.
- [79] P. Darachai, S. Chutipaijit, and K. Sompornpailin, "Carbon sources and supporting materials in callus induction effects on regeneration of *indica* rice (*Oryzasativa* L. cv. RD6 and RD15)," In the 8th international symposium on biocontrol and biotechnology, Thailand, pp. 266-272, 2010
- [80] Z. Hussain, M. H. Khan, R. Bano, H. Rashid, and Z. Chaudhry, "Protocol optimization for efficient callus induction and regeneration in three Pakistani rice cultivars," Pak. J. Bot., vol. 42(2), pp. 879-887, April 2010.
- [81] P. Sivakumar, Y. S. Law, C. L. Ho, and J. A. Harikrishna, "High frequency plant regeneration from mature seed of elite, recalcitrant Malaysian *indica* rice (*Oryzasativa* L.) CV. MR 219," *Acta Biol. Hung.*, vol. 61(3), pp. 313-321, September 2010.
- [82] S. H. Wani, A. A. Lone, T. Da Silva, and S. S. Gosal, "Effects of NaCl stress on callus induction and plant regeneration from mature seeds of rice (*Oryzasativa* L.)," *Asian Australas. J. Plant Sci. Biotechnol.*, vol. 4(1), pp. 57-61, March 2010.
- [83] E. Shahsavari, "Contribution of sorbitol on regeneration of embryogenic calli in upland rice," *Int. J. Agric. Biol.*, vol. 13, pp. 838-840, January 2011.
- [84] D. Verma, R. Joshi, A. Shukla, and P. Kumar, "Protocol for *in vitro* somatic embryogenesis and regeneration of rice (*Oryzasativa* L.)," *Indian J. Exp. Biol.* vol. 49, pp. 958-963, December 2011.
- [85] S. H. Wani, G. S. Sanghera, and S. S. Gosal, "An efficient and reproducible method for regeneration of whole plants from mature seeds of a high yielding *indica* rice (*Oryzasativa* L.) variety PAU 201," *New Biotechnol.*, vol. 28(4), pp. 418-422, July 2011.
- [86] A. R. Zuraida, B. Naziah, Z. Zamri, and S. Sreeramanan, "Efficient plant regeneration of Malaysian *indica* rice MR 219 and 232 via somatic embryogenesis system," *Acta Physiol. Plant.*, vol. 33(5), pp.1913-1921, September 2011.
- [87] M. J. Alam, M. Imran, L. Hassan, M. H. Rubel, and M. Shamsuddoha, "*In vitro* regeneration of high yielding *indica* rice (*Oryzasativa* L.) varieties," *J. Environ. Sci. Nat. Resour.*, vol. 5(1), pp. 173-177, August 2012.
- [88] A. Libin, P. J. H. King, K. H. Ong, J. K. Chubo, and P. Sipeen, "Callus induction and plant regeneration of Sarawak rice (*Oryzasativa* L.) variety Biris," *Afr. J. Agric. Res.*, vol. 7(30), pp. 4260-4265, August 2012.
- [89] Z. Yinxia, and S. Te-Chato, "Callus induction and plantlet regeneration from mature embryos of *indica* rice (*OryzaSativa* L.) cultivar Kra Dang Ngah. *J. Agric. Tech.*, vol. 8, pp. 2423-2433, November 2012.
- [90] F. A. Joyia, and M. S. Khan, "Scutellum-derived callus-based efficient and reproducible regeneration system for elite varieties of *indica* rice in Pakistan," *Int. J. Agric. Biol.*, vol. 15(1), pp. 27-33, February 2013.
- [91] S. R. Krishnan, A. M. Priya, and M. Ramesh, "Rapid regeneration and ploidy stability of 'cv IR36' *indica* rice (*Oryzasativa* L.) confers efficient protocol for *in vitro* callus organogenesis and *Agrobacteriumtumefaciens* mediated transformation," *Bot. Stud.*, vol. 54(1), pp. 1-12, December 2013.

- [92] M. A. Mannan, T. C. Sarker, M. T. Akhter, A. H. Kabir, and M. F. Alam, "Indirect plant regeneration in aromatic rice (*Oryzasativa* L.) var. Kalijira and Chinigura," *Acta Agric. Slovenic.*, vol. 101(2), pp. 231-238, August 2013.
- [93] Z. Yin Xia, and S. Te-Chato, "Improved plantlet regeneration systems in *indica* rice (*Oryzasativa* L.) landrace Hom Kra Dang Ngah," *Int. J. Agric. Tech.*, vol. 9, pp. 1641-54, October 2013.
- [94] R. Bhuiyan, M. A. Miah, S. K. Shakil, M. Ferdous, and S.H. Prodhan, "Comparison of callus initiation and regeneration frequency for two submerge tolerant rice (*Oryzasativa*)," *J. Pharmacy Biol. Sci.*, vol. 9(1), pp. 74-78, January 2014.
- [95] I. D. Barbosa Cepeda, and A. Chaparro Giraldo, "Optimization of an *in vitro* regeneration system for Colombian *indica* rice varieties," *Rev. Colomb. Biotecnol.*, vol. 16(2), pp. 19-29, December 2014.
- [96] M. M. Islam, M. E. Haque, M. A. Islam, B. Sikdar, and M. Khalekuzzaman, "Establishment of an efficient protocol for *in vitro* callus induction and regeneration system using mature embryo in elite rice (*Oryzasativa* L.) cultivars," *Plant Biol.*, vol. 4(4), pp. 09-20, July 2014.
- [97] Z. Y. Roly, M. M. Islam, M. P. E. Shaekh, M. S. I. Arman, S. M. Shahik, D. Das, M. M. E. Haamem, and M. Khalekuzzaman, "*In vitro* callus induction and regeneration potentiality of aromatic rice (*Oryzasativa* L.) cultivars in differential growth regulators," *Int. J. Appl. Sci. Biotechnol.*, vol. 2(2), pp. 160-167, June 2014.
- [98] E. Toppo, M. Ramakrishnan, S. A. Ceasar, K. Sivasankaran, A. Premkumar, and S. Ignacimuthu, "Regeneration from mature scutellum explants of rice variety IR64 (*Oryzasativa* L.) through direct and indirect organogenesis. *J. Glob. Agric. Ecol.*, vol. 1(1), pp. 1-9, 2014.
- [99] H. Ghobeishavi, E. D. Ulihaie, S. S. Alavikia, and M. Valizadeh, "Study of factors influencing somatic embryogenesis in rice (*Oryzasativa* L.)," *Int. J. Adv. Biol. Biom. Res.*, vol. 3(1), pp. 43-50, January 2015.
- [100] M. R. Hossain, N. Akter, U. Mondal, R. C. Dey, and L. Hassan, "Investigating the *in vitro* regeneration potentiality of three high yielding *indica* rice (*Oryzasativa* L.) varieties," *Br. Biotechnol. J.* vol. 9(3), pp. 1-13, September 2015.
- [101] M. Islam, Z. Y. Roly, Z. Naim, and M. Khalekuzzaman, "*Agrobacterium* -mediated genetic transformation and regeneration in elite rice (*Oryzasativa* L.) cultivar BRRI dhan56," *Afr. J. Biotechnol.*, vol. 14(31), pp. 2415-2423, August 2015.
- [102] R. Mahajan, and S. Sharma, "Comparison of regeneration efficiency of three different genotypes of basmati rice under *in vitro* condition," *IJBTT.*, vol. 11(1), pp. 16-24, June 2015.
- [103] T. N. Tran, and N. Mishra, "Effect of antibiotics on callus regeneration during transformation of IR 64 rice," *Biotechnol. Rep.*, vol. 7, pp. 143-149, September 2015.
- [104] A. R. Vennapusa, R. S. Vemanna, B. H. R. Reddy, K. C. Babitha, K. Kiranmai, A. Nareshkumar, and C. Sudhakar, "An efficient callus induction and regeneration protocol for a drought tolerant rice *indica* genotype AC39020," *Plant Sci.*, vol. 3, pp. 248-254, September 2015.
- [105] V. N. Juturu, G. K. Mekala, M. Garlandinne, P. C. O. Reddy, and A. C. Sekhar, "Optimization of *in vitro* regeneration protocol for a popular *indica* rice (*Oryzasativa* L. cv. Swarna)," *Ann. Plant Sci.*, vol. 5, pp. 1395-1401, July 2016.
- [106] A. Poeaim, S. Poeaim, R. Poraha, S. Pongjaroenkit, and P. Pongthongkam, "Optimization for callus induction and plant regeneration from mature seeds of Thai rice variety: Nam Roo (*Oryzasativa* L.)," *J. Biosci. Bioeng.*, vol. 4(5), pp. 95-99, 2016.
- [107] R. Poraha, A. Poeaim, S. Pongjaroenkit, and P. Pongthongkam, "Callus induction and plant regeneration on optimization of the culture conditions in Jow Haw rice (*Oryzasativa* L.)," *J. Agric. Sci. Technol.*, vol. 12(2), pp. 241-248, February 2016.
- [108] S. S. R. Sankepally, and B. Singh, "Optimization of regeneration using differential growth regulators in *indica* rice cultivars," *3 Biotech*, vol. 6(1): 1-7, June 2016.
- [109] I. B. M. Artadana, G. B. F. Suhono, P. H. Hardjo, M. G. M. Purwanto, and K. Supaibulwatana, "Plant regeneration induced from mature embryo-derived callus of Balinese red rice (*Oryzasativa* cv. Barak Cenana)," *Bali Med. J.*, vol. 6(3), pp. 12-17, August 2017.

- [110] A. Chakraborty, H. Hoque, M. N. Hasan, F. Akter, S. Suhani, Z. F. Joy, and J. Akther., “Effect of different concentrations of plant growth hormones for *in vitro* regeneration of rice varieties BRR1 Dhan 28 and BRR1 Dhan 29,” *Int. J. Sci.: Basic Appl. Res.*, vol. 33(2): 26-33, 2017.
- [111] S. B. Mostafiz, A. Wagiran, N. S. Johan, and N. S. A. Zulkifli, “The effects of temperature on callus induction and regeneration in selected Malaysian rice cultivar *indica*,” *Sains. Malays.*, vol. 47, pp. 2647-2655, November 2018.
- [112] A. Trunjaruen, R. A. S. O. Sayam, P. Maneerattanarungroj, and W. Taratima, “Effects of cultivation media on *in vitro* callus induction and regeneration capabilities of Pakaumpuel rice (*Oryzasativa* L.), Thai rice landrace,” *Walailak J. Sci. Technol.*, vol. 17(1), pp. 37-46, November 2020.
- [113] M. N. M. Khan, M. M. Islam, M. S. Islam, and M. I. Uddin, “Studies on *in vitro* response to callus induction and gene transfer technique of five high yielding *indica* rice varieties,” *J. Sci. Agric.*, vol. 3, pp. 41-45, May 2019.
- [114] Y. L. Lee, J. O. Abdullah, Y. W. Chien, R. Sekeli, C. K. Tan, J. Y. Loh, and K. S. Lai, “Effects of lignosulfonates on callus proliferation and shoot induction of recalcitrant *indica* rice,” *Sains Malays.*, vol. 48(1), pp. 7-13, June 2019.
- [115] S. Paul, and A. Roychoudhury, “Comparative analyses of regeneration potentiality of eight indigenous aromatic *indica* rice (*Oryzasativa* L.) varieties,” *Int. J. Sci. Res. Biol. Sci.*, vol. 6(1), pp.55-64, February 2019.
- [116] P. Rakshana, R. Valarmathi, and M. Raveendran, “Optimization of tissue culture protocol for rapid regeneration of traditional therapeutic rice genotype Kavuni,” *Electron. J. Plant Breed.*, vol. 10(2), pp. 334-340, June 2019.
- [117] R. Hasan, M. S. U. Bhuiyan, G. Deb, M. M. A. Dina, and S. Sultana, “Investigating the *in vitro* regeneration potential of selected local rice cultivars in Bangladesh,” *Fundam. Appl. Agric.*, vol. 5(3), pp. 353-360, September 2020.
- [118] S. Shweta, S. Varanavasiappan, K. K. Kumar, D. Sudhakar, L. Arul, and E. Kokiladevi, “Protocol optimization for rapid and efficient callus induction and *in vitro* regeneration in rice (*Oryzasativa* L.) cv. CO 51,” *Electron. J. Plant Breed.*, vol. 11(03), pp. 755-759, September 2020.
- [119] S. Sundararajan, H. P. Sivakumar, S. Nayeem, V. Rajendran, S. Subiramani, and S. Ramalingam, “Influence of exogenous polyamines on somatic embryogenesis and regeneration of fresh and long-term cultures of three elite *indica* rice cultivars,” *Cereal Res. Commun.*, vol. 49(2), pp. 245-253, June 2021.
- [120] M. S. A. Banu, B. Ahmed, S. Parveen, M. H. U. Rashid, and K. M. K. Huda, “*Agrobacterium*-mediated genetic transformation of rice var. BRR1 Dhan 58,” *Plant Tissue Cult Biotechnol.*, vol. 31(1), pp. 71-80, June 2021.
- [121] N. Carsono, E. Juwendah, L. Liberty, S. Sari, F. Damayanti, and M. Rachmadi, “Optimize 2, 4-D concentration and callus induction time enhance callus proliferation and plant regeneration of three rice genotypes,” *Biodiversitas: J. Biol. Divers.*, vol. 22(7), pp. 2555-2560, June 2021.
- [122] M. N. Hasan, F. H. Bhuiyan, H. Hoque, N. A. Jewel, M. Ashrafuzzaman, and S. H. Prodhan, “The effect of plant growth regulators (PGRs) on efficient regeneration of 12 recalcitrant *indica* rice (*Oryzasativa* L.) genotypes,” *Am. J. Biochem. Biotechnol.*, vol. 17(2), pp. 148-159, March 2021.
- [123] S. K. Tripathy, “Optimization of culture variables for efficient callus induction and rapid plant regeneration in zinc rich rice (*Oryzasativa* L.) cv. Chittimuthyalu,” *J. Appl. Biol. Biotechnol.*, vol. 9(4), pp. 1-9, July 2021a
- [124] S. K. Tripathy, “Genotypic response to matured embryo derived callus induction and plant regeneration in high yielding upland rice (*Oryzasativa* L.),” *Appl. Biol. Res.*, vol. 23(3), pp. 261-267, July 2021b
- [125] M. Sutradhar, “*Agrobacterium*-mediated transformation of *indica* rice varieties popularly grown in West Bengal,” (Doctoral thesis, Bidhan Chandra Krishi Viswavidyalaya), 2022.