Review article

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Effects and Quantity Ranges Of Some Auxins On Embryogenic Callus Induction From Upland Rice Cultivars: An Overview

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ABSTRACT

Embryogenic callus induction from indica rice sub-species, upland cultivars has remain a challenging task, but plant growth regulators (PGRs) have been found to play a vital role in enhancing the production. Hence, solving this tricky problem and achieving optimum embryogenic callus induction from this special type of rice is a welcome development as a food security concern, while proper examination of the appropriate PGRs is the only solution. Here we reviewed on two auxins-PGRs that promote the embryogenic callus induction from upland rice, viz., 2,4-D and NAA with their recommendable quantity ranges. 2,4-D and NAA were the most commonly employed and discovered best growth hormones in PTC irrespective of explant in all cereal plants. They are found the most suitable hormones and positively upgrade the production of reproducible callus. Classical analyses implies that medium supplemented with such PGRs provides necessities for quality callus from upland rice. Evidences indicated that callus induction or somatic embryogenesis from matured seeds of upland rice is persuade by 2,4-D between 1.0 – 2.5 mg/L concentrations. Whereas for NAA, to obtain its desired effects on upland rice callus induction it must be fortified in concentrations ranging from 0.5 mg/L to 10 mg/L. But convincingly, 0.5 – 5.0 mg/L NAA with other PGR like 2,4-D would be more proper. Hence, extensive studies of these hormones and others will provide insights on their potentialities to embryogenic callus induction and probably identify more of their roles in PTC analysis.

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Introduction

Rice or its botanical name Oryza sativa L. is divided into three sub-species which includes indica, japonica and javonica [1]. Indica sub-species, precisely upland, is planted and grown in dry-land, rain-feeding or short irrigation condition [2]. Farming of these rice varieties may lessen the laborious irrigation, save plenty of water and probably reduce water pollution. Up- land rice is a special rice type which comprises almost 80% of the world cultivated rice and found in many Asian nations [2-5]. For example, Malaysia has diverse number of upland rice cultivars [6], mostly cultivated in Sabah and Sarawak [7].

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Regarding all these advantages over wetland rice varieties, upland rice contributes only 12% of global rice production [2, 3].

Evidences exist to show that upland rice has the potential to be explored as alternative com- pare to wetland rice [2]. Whereas, research on upland rice improvement has gained little at- tention because of its unstable grain yield which occurred due to poor agronomy management practices and lack of application of suitable nutrient as described by Musa, Azemi [8]. Thus, to increase the production of this rice species; calluses induction and regeneration (biotech- nology approach) through plant tissue culture (PTC) as well as genetically (molecular ap- proach) improve the cultivars via biotransformation are the alternative solutions to this pecu- liar problem. PTC or plant micro-propagation describe the aseptic culture of explant under control physical and chemical condition in vitro [9, 10]. It is considered the best approach for demonstrating the plant cells totipotency and its utilization for various practical solicitations [11]. Also, the case is not different in Oryza sativa L. as earlier discovered by Koetje, Grimes [12], Croughan and Chu [13] and Oinam and Kothari [14].

In plant tissue culture (PTC), the explant can either be mature or immature seeds, leaves, buds, node or tips of root [15]. The explant callus induction, growth and development and morphogenic response in tissue culture depends on genotype, explant type, environment, cul- ture medium and plant growth regulators (PGRs). According to Khanna and Raina [16], Oza- wa, Ling [17] Gairi and Rashid [18], Karthikeyan, Pandian [19], Meneses, Flores [20], [Bho- jwani and Dantu [11], 21], embryogenic callus induction massively rely on PGRs, medium composition (as they can be easily manipulated) along site with explant genotype and culture environment. Interestingly, media supplemented with PGRs provides necessities for quality and reproducible calli [22]. Embryogenic calli production is the prerequisite for achievable regeneration and remain problematic in indica sub-species such as upland rice. However, proper examination of the appropriate medium and its supporting supplement(s) that may in- duce qualitative and quantitative embryogenic calli is significant. Reports have indicated that optimizing the culture media for indica culture using PGRs, precisely auxins have yielded a recommendable outcome [3, 23-28]. Therefore, the aim of this review is to provide an insight overview of two most commonly used auxins namely; 2,4-Dichlorophenoxyacetic acid (2,4- D) and Naphthaleneacetic acid (NAA) and their recommendable quantity ranges that make embryogenic callus from

upland rice cultivars.

Plant Growth Regulators (PGR) and Their Classification

As far as PTC is concerned, the important point is that, due to the plasticity and totipo- tency of the explant [29, 30]. Precise media manipulations can be practice to direct embryo- genic callus development in culture. Manipulation or optimization of medium are either from carbon source, amino acids basis, vitamin or growth regulators [31]. Reports have shown that phytohormones (PGRs) are the critical constituents for determining the target callus in addi- tion to the developmental pathway of the plant cells [32].

effects of hormones (PGRs) have been widely investigated in tissue culture tech- nology. They are widely considered as essential parameter(s) in determining the success of embryogenic callus induction [33]. Trejo-Tapia, Amaya [34] communicated that, combina- tions of hormone type and its various concentration can greatly promote the morphogenetic development leading to calluses production. The employed PGRs in plant tissue culture analysis are classified into five (5) main classes, they include; Auxins, Cytokinins, Gib- berellins, Abscisic acid and Ethylene.

The first four class of PGRs mentioned above are used so open, whereas the first two are mostly fortified in upland rice analysis. According to Skoog and Miller [35], auxin and cytokinin ration determine the type and extent of organogenesis in plant cell cultures. Never- theless, pieces of reports described the vitality of auxins as a constituent in upland rice culture and dedifferentiation processes in in vitro cultures. The most commonly used auxins in upland rice culture are the synthetic analogues, viz, 2,4-Dichlorophenoxyacetic acid (2,4-D) and Naphthaleneacetic acid (NAA). Therefore, in order to obtain embryogenic callus from upland rice specimen, auxin(s) hormones should be added into the medium. Although their rations required are not universally same. Bhaskaran and Smith [36] reported that there is erraticism on the quantity ratio of such hormones required for callus induction among genera, species, sub-species and cultivars.

2,4-Dichlorophenoxyacetic acid

In monocotyledonous plants, the use of auxins as growth regulator or promoters of cell indifferentiation is absolutely possible. These enhances induction of indirect somatic embryo- genesis as reconfirmed by Meneses, Flores [20], Karthikeyan, Pandian [19], [31]. Auxins have significant effect by inducing embryogenic competence on the scutellar

cells of rice seeds, leaves and other organs. Rice cultivar's genotypic difference shows diversification in their specificity towards response to auxins concentration [18, 37]. Among the various auxins, 2,4-D is the best regulator discovered so far. 2,4-D is the most employed in tissue analysis irrespective of explant in all cereal species. This synthetic auxin provides a desirable and effi- cient embryogenic calli when supplemented in upland rice tissue culture regardless of the medium type. 2,4-D has chemical formulae C8H6Cl2O3 and schematic diagram as shown in figure 1.

Zhu, Sun [38] revealed on the suitability of 2,4-D on embryogenic callus induction. Others have recommended 2,4-D as an in vitro plant regulator for development of callus in upland rice culture and hence always employed in medium either; singly, in combination with other auxins or cytokinin [33]. Endress and Endress [39] reported that, 2,4-D generate DNA hypermethylation which uphold cells in active mitotic stage and therefore, in favour of em- bryonic phase.

Previous reports indicated that embryogenic calli production from indica sub-species face a major set-back due to its low tissue response [24, 40]. But recent evidences demon- strated that callus induction or indirect somatic embryogenesis from matured seeds of upland rice is persuade by 2,4-D at different concentrations [31]. Thus, the concentration of the hor- mone used, the culture period and sub-culture time control the responses and virtually recon- version of embryos-to plants. However, distinct 2,4-D responses has been observed as a func- tional enhancer of callus production from upland genotype; since they are characterized for having difficulties in tissue responsive [2-4, 20, 24]. The use of 2,4-D in high concentration is necessary in some upland varieties as they provide positive responses towards embryogenic calluses production, but simultaneously they exert an inhibitory effect on plant regeneration because the hormone residues remain within the embryo cells.

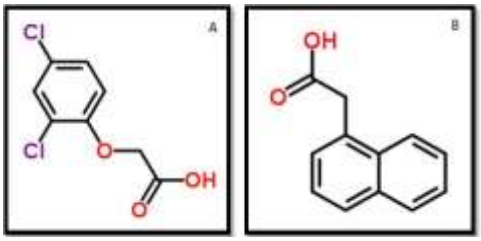

Fig 1 Schematic representation of (A) 2,4-D and (B) NAA growth regulator

Shahsavari, Maheran [2] discovered that using 2,4-D alone at 2 mg/L gave the earliest and optimum callus induction (84%). This is consistent with other earlier reports that higher callus induction were observed using 2 mg/L 2,4-D [41]. The highest percentage of callus in- duction (85%) were obtained on media containing 5 mg/L 2,4-D [42]. In another report by Shahsavari [43] reveal that 2 mg/L 2,4-D in combination with other hormones gave up to 95% callus induction frequency. This concentration is almost the same with that of Ahmad, Johan

[4] and Karthikeyan, Pandian [19] whose reported that 2.5 mg/L 2,4-D gave optimal induction in Malaysian upland rice. Evenly, 2.5 mg/L 2,4-D plus NAA fortified in another Malaysian upland rice culture, optimum induction were achieved [44]. Also, 3 mg/L 2,4-D was reported and optimal callus induction (90%) was obtained [5]. Conclusively, many reports recom- mended the use of 1.0 – 2.5 mg/L 2,4-D for callus induction from upland rice tissue [18, 19, 33].

α-Naphthalene-acetic acid

NAA is another essential synthetic auxin hormone that is routinely used for the vege-tative propagation of plants. It is widely used in agriculture for various purposes and for tissue culture research [2, 24]. NAA is an organic compound with the formula $C_{10}H_7CH_2CO_2H$ [schematic diagram (fig. 1b)] and auxin family that prevents premature dropping and thinning of fruits from stems. NAA has been shown to greatly increase cellulose fiber formation in plants when paired with another phytohormone called gibberellic acid. This plant regulator plays a role in both embryogenic callus production and regeneration from upland cultivars as communicated by Aananthi, Anandakumar [45] and Din, Ahmad [5]. The effect of NAA on callus growth is greatly reliant on the time of applying and its concentration. The hormone is only slightly toxic when applied at higher concentration. Thus, this is to understand that in- creased amounts of NAA can actually have negative effects, however, cause growth inhibition to the development of crops such as rice.

For plant growth, NAA is used after 4-weeks to stimulates shoot growth, while full- time use limits the plant growth. When used in 4-week pulse, adventitious root growth is greatly increased. In micropropagation NAA is typically added to a media containing essential nutrients for explant survival. It is added to help induce embryogenic callus induction [26, 43], shoot and root development [24, 33, 45].

For NAA to obtain its desired effects in upland rice callus induction, it must be forti- fied in concentrations ranging from 0.5 mg/L to 10 mg/L as recommended by Zuraida, Naziah [26]. Many have used the concentration between 0.5-5.0 mg/L, but so far it is only Zuraida, Naziah [26] and Zuraida, Zulkifli [42] that obtained maximum callus production from upland rice at 10 mg/L of NAA. Whereas, high concentration may leads to optimum induction from upland rice, nonetheless depend on genotype. Going by all the practiced concentrations, 10 mg/L was the highest used for upland rice callus induction which may affect the quality of the callus or even shoot. As earlier discussed, NAA at high concentration is toxic, therefore the use of maximum concentration (10 mg/L) may be problematic to crop quality. As less as 1 mg/L can make optimum callus from upland rice (Malaysian cultivar) [44]. Also 3 mg/L proved to be beneficial for callus induction [46], but all depend on the genotype. Using a slightly high concentration around 5 mg/L as described by Shahsavari [43] is also appropriate. Convincingly, supplementing 0.5 – 5.0 mg/L NAA with other PGR like 2,4-D for callus in- duction from upland rice it would be more proper.

Conclusion

Embryogenic callus induction is the prerequisite for micropropagation, regeneration and ge- netic transformation. Achieving optimum embryogenic callus induction from upland rice cul- tivars is a welcome development as a food security concern. 2,4-D and NAA were found the most useful auxin growth hormones and positively upgrade the production of reproducible callus. There is a need for appropriate utilization of these PGRs at a certain range of concen- tration. Usually, tissue culture of upland rice without any of the reviewed hormones yields no embryogenic callus. Therefore, this review achieved the aim of providing useful information on such auxins (2,4-D and NAA) and their concentration range that would yield maximum embryogenic callus from upland rice. However, extensive studies of these plant regulators will provide more significant insights to their potential in callus induction and identify more of their roles in PTC analysis.

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