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RICE (*ORYZA SATIVA* L.) ANDROGENESIS *IN VITRO*

Rice (Oryza sativa L.) is a cereal crop cultivated mainly in tropical and even some subtropical countries and provides food for more than half of the world's population. Nowadays, haploid biotechnology (gynogenesis and androgenesis) is used in breeding practice as one of the tools to improve rice, preferably in vitro androgenesis. The method of in vitro cultivation of male gametophyte is one of the promising biotechnological approaches in crop breeding research, including rice. This method is based on the biological phenomenon of androgenesis – formation of haploid regenerant plant from anther and microspore cells, whose development switches from gametophytic to sporophytic pathway. However, the wide application of these technologies is limited by the existing two problems associated with the genotype-dependence of plant regeneration and formation of albino plants in many cases in in vitro male gametophyte culture of rice. The need to eliminate these restrictions has been and remains. This requires continued research in this direction to develop and optimize the technology of cultivation, pre-treatment of anthers and components of the nutrient medium, universal for all rice genotypes. This review considers a number of factors that affect the efficiency of rice androgenesis in vitro.

Key words: Rice (Oryza sativa L.), androgenesis in vitro, anthers culture, isolated microspores culture, limiting factors.

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In *vitro* жағдайындағы күріш (*Oryza sativa* L.) андрогенезі

Күріш (Oryza sativa L.) дүние жүзі халқының жартысынан астамын азық-түлікпен қамтамасыз ететін маңызды дәнді дақыл болып табылады, кейбір тропиктік, тіпті субтропиктік елдерде өсіріледі. Қазіргі уақытта гаплоидты биотехнология (гиногенез және андрогенез) селекциялық тәжірибеде күрішті жақсарту құралдарының бірі ретінде in vitro-ғы андрогенезде қолданылады. In vitro андрогенездің дәстүрлі селекция әдістерімен салыстырғанда артықшылығы генотипте ата-аналық формалардың экономикалық құнды белгілерін сақтайтын 1-ші ұрпақтың гомозиготалы тұрақты будандарын аз уақыт аралығында алу мүмкіндігі болып табылады. Аталық гаметофитті in vitro жағдайында өсіру әдісі ауыл шаруашылығы дақылдарын, оның ішінде күрішті зерттеудегі перспективті биотехнологиялық тәсілдердің бірі болып табылады. Бұл әдіс андрогенездің биологиялық құбылысына негізделген – гаметофитті жолдан спорофитті жолға ауысатын тозаң және микроспора жасушаларынан гаплоидты регенерант өсімдіктің түзілуі. Алайда, бұл технологияларды кеңінен қолдану өсімдіктердің регенерациясының генотипке тәуелділігімен және көптеген жағдайларда in vitro күріштің тозаң және микроспоралы дақылында альбинос өсімдіктерінің қалыптасуымен байланысты екі мәселемен шектеледі. Бұл шектеулерді жою қажеттілігі болды және болып қала береді. Бұл күріштің барлық генотиптері үшін әмбебап өсіру технологиясын, тозаңдарды алдын ала өңдеу және қоректік ортаның құрамын әзірлеу және оңтайландыру үшін осы бағыттағы зерттеулерді жалғастыруды талап етеді. Бұл шолуда in vitro жағдайындағы күріш андрогенезінің тиімділігіне әсер ететін бірқатар факторлар қарастырылады.

Түйін сөздер: күріш (Oryza sativa L.), андрогенез in vitro, тозаңқап дақылдау, оқшауланған микроспораларды дақылдау, шектеуші факторлар.

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Андрогенез риса (*Oryza sativa* L.) *in vitro*

Рис (Oryza sativa L.) – важная зерновая культура, обеспечивающая питанием более половины населения земного шара и выращиваемая главным образом в тропических и даже в некоторых субтропических странах. В настоящее время гаплоидная биотехнология (гиногенез и андрогенез) используется в селекционной практике как один из инструментов улучшения риса, преимущественно андрогенеза in vitro. Метод культивирования in vitro мужского гаметофита является одним из перспективных биотехнологических подходов в селекционных исследованиях сельскохозяйственных культур, в том числе риса. Преимущество андрогенеза in vitro по сравнению с традиционными методами селекции заключается в возможности быстрого получения гомозиготных константных гибридов 1-го поколения, сохраняющих в генотипе хозяйственно-ценные признаки родительских форм. Этот метод основан на биологическом явлении андрогенеза – образовании из пыльников и клеток микроспор гаплоидного растения-регенеранта, развитие которого переключается с гаметофитного пути на спорофитный. Однако широкое применение этих технологий ограничивается существующими двумя проблемами, связанными с генотипической зависимостью регенерации растений и формированием во многих случаях растений-альбиносов в культуре пыльников in vitro и микроспор риса. Необходимость устранения этих ограничений была и остается. Это требует продолжения исследований в этом направлении по разработке и оптимизации технологии выращивания, предварительной обработки пыльников и состава питательной среды, универсальной для всех генотипов риса. В данном обзоре рассмотрен ряд факторов, влияющих на эффективность андрогенеза риса in vitro.

Ключевые слова: рис (Oryza sativa L.), андрогенез in vitro, культура пыльников, культура изолированных микроспор, лимитирующие факторы.

Introduction

Haploid plants in rice were first discovered by Morinaga and Fukushima in 1931[1], and in 1968 Niizeki and Oono obtained the first haploid plants in rice anther culture [2].

Since then, there have been many studies aimed at improving the efficiency of various aspects of the male gametophyte in rice and the use of dihaploids in breeding. Currently, there is a lot of information in the literature about rice androgenesis *in vitro* [3-8]. The purpose of this article was to analyze the literature data devoted to the study of a number of factors affecting the processes of androgenesis *in vitro* in rice.

Androgenesis *in vitro* is the reprogramming of the development of the male gametophyte from the gametophytic to the sporophytic path of development with the formation of regenerated plants with a haploid set of chromosomes. The use of androgenesis significantly accelerates the production of homozygous constant lines carrying economically valuable traits and representing valuable material for selection [3-8].

At the same time, there are problems that reduce the effectiveness of this method and its widespread use in breeding practice. However, the wide application of these technologies is limited by the existing two main problems associated with the genotypic dependence of plant regeneration and the formation of albino plants in many cases in rice anther and microspore cultures in vitro. Until now, the need to eliminate these restrictions has been and remains. This requires continued research on the optimization of cultivation technology, anther pretreatment and components of the nutrient medium.

Techniques of androgenesis in vitro

There are two approaches to obtaining haploid plants by inducing androgenesis under aseptic conditions. The first is anther culture, in which isolated anthers are cultured on a solid nutrient medium. Second, microspores culture, in which pollen grains are released from the anther, and then cultivated on a liquid nutrient medium. Thus, in this article, the term androgenesis *in vitro* is used in a broad sense, including the culture of anthers and microspores.

Factors affecting the androgenesis of rice in vitro

An analysis of the literature data revealed that the success of the cultivation of rice anthers and

microspores is influenced by numerous biological, chemical and physical factors.

Among the biological factors, androgenesis of rice *in vitro* is strongly influenced by the donor plant genotype, its physiological state and developmental stage of the male gametophyte.

Among the chemical factors influencing the success of androgenesis *in vitro* are the mineral composition of nutrient media, growth stimulants, vitamins and organic additives. Physical factors include the state (type) of the nutrient medium, temperature and radiation pretreatment of the planting material, temperature and light conditions of cultivation.

Biological factors

Donor plant genotype. The genotype of the initial material is one of the important factors determining the positive results of cultivating isolated anthers and microspores. Therefore, the identification and selection of genotypes responsive to cultivation conditions is a primary task [9-12].

Due to the strong dependence on the genotype, some researchers suggest that the starting material for androgenesis should be crossed with well-regenerating varieties [13, 14]. Since this is not always possible, an alternative solution is to select optimal cultivation conditions for each individual rice genotype [15].

Donor plant physiological state. The physiological state of plants at the time of cultivation is one of the key factors influencing the induction of androgenesis *in vitro* [3-8, 11].

Healthy donor plants, their shoots and ears are the first important factors in the implementation of accelerated production of dihaploids. Two commonly used methods (controlled conditions in a greenhouse or phytotron chamber; favorable field conditions in the nursery) help breeders to work in this direction. Controlled light and temperature conditions (greenhouse, phytotron chamber) allow growing donor plants throughout the year [16].

Some researchers prefer materials grown in the field for their work. Generally, the capacity for androgenesis *in vitro* of anthers and microspores from donor plants harvested from the field is significantly superior to that of plants grown under controlled conditions. They form more shoots with a developed spike, large anthers and microspores inside the anthers [16].

This is mainly, as experiments show, the number of viable microspores formed in anthers depends on the donor plant physiological state and on the nutritional status of anther tissues [11].

Phase of male gametophyte development. The phase of development of pollen grains in anthers plays a decisive role in the induction of morphogenetic processes up to the production of haploid regenerants [3-8, 11]. Therefore, before cultivating anthers, it is necessary to determine the development stage of the male gametophyte. The stage of microspore development can be determined by morphological and cytoembryological characteristics [3, 11]. It was found that the level of starch content in microspores is the best cytological marker in comparison with other morphological features [12].

For rice, the best stage for the induction of embryogenesis is the middle or late single-core stage of microspore division [8, 17, 18]. Probably, microspores at this stage are poorly differentiated and have an increased morphogenetic potential for the induction of callusogenesis, embryogenesis and organogenesis and recovering of the whole plant [18].

It is important to note that in many cases, mononuclear microspores from the early to late stages of maturation of the male gametophyte are more responsive to the induction of androgenetic processes. However, the most appropriate stage of microspore development may differ for different genotypes.

Chemical factors

Components of the nutrient medium. A very important factor affecting androgenesis *in vitro* is the components of the nutrient media. In particular, the concentration and combination of phytohormones or plant growth regulators (PGR) play a certain role. In various experiments, the addition of the following components is used: macro- and microelements, vitamins, carbohydrates, amino acids, activated carbon, various organic additives. Modified Murashige and Skoog [19], Gamborg B5 [20], N_6 [21] and others basic nutrient media are used for the cultivation of isolated anthers and microspores [3-8].

Carbohydrates are necessary in media for the cultivation of anthers and microspores as an energy source and as osmotic agents [18]. The use of one or another group of carbohydrates greatly influences the response of cultivated anthers. The correct choice of the carbon source greatly influences the reaction of the anther. A number of researchers have noticed that the use of maltose instead of sucrose has a positive effect on increasing the efficiency of induction of androgenesis in rice in vitro [11, 22].

Sucrose has a toxic effect on androgenesis *in vitro*. Since, when sucrose is added to the nutrient

medium, it breaks down with the formation of glucose and fructose. As is known, microspores are very sensitive to fructose [11].

The type and concentration of PGR in the induction nutrient medium is very important for changing the pathway of development of microspores from gametophytic to sporophytic. It has been established that not only the combination of growth regulators, but also the auxin/cytokinin balance play a significant role in the regulation of in vitro androgenesis in rice [3-8].

Physical factors

The physical state of the nutrient media. Usually, rice anthers are cultivated on solid nutrient media. However, there is evidence that cultivation on a solid medium leads to increased necrosis of the anther tissue. Liquid nutrient media are often used to monitor anther and micropore that have limited access to nutrients and growth regulators [3-6, 8, 11, 12]. Agar is widely used as a gelling agent of solid nutrient media. It has also been shown that the replacement of agar with gelrite or starch increased the efficiency of androgenesis *in vitro* [11, 23].

Pretreatment conditions of rice anthers for androgenesis in vitro. According to literature data, pre-stress treatment of anthers plays a key role in reprogramming microspores to the path of sporophytes, which practically means the induction of embryogenesis *in vitro*.

It has been established that pre-treatment of planting material at low positive temperatures has a positive effect on the induction of androgenesis in rice. By inhibiting the processes of destruction of male gametophyte cells [3-6, 11, 24]. At the same time, it was noted that sensitivity to cold treatment varies among different genotypes. It has been shown that in rice, 8 days of cold stress at 8-10°C stimulates the induction of morphogenetic processes [25], and 11 days of pretreatment reduces the yield of albino seedlings [26]. In some cases, a combination of osmotic stress with cold pretreatment is used to induce androgenesis *in vitro* in rice. It was found that changes in the level of endogenous auxin under osmotic stress caused by exposure to low positive temperatures increase the induction of callusogenesis [27]. In rice, pretreatment with cold can be partially replaced by sugar starvation to obtain the frequency of embryogenesis and regeneration of seedlings during androgenesis *in vitro* [28, 29].

The use of low doses of radiation improved callus induction and plant regeneration in resistant varieties but increasing the radiation dose had the opposite effect. It has been noted that the effectiveness of radiation exposure varies among different rice genotypes [32] and depended on the stage of development of the male gametophyte at the time of inoculation [33].

Conclusion

The effectiveness of androgenesis *in vitro* depends on biological, chemical and physical factors, including: the genotype of the mother plants, the physiological state of the starting material, the degree of maturity of the male gametophyte, the composition and type of nutrient media, exposure to low temperatures and radiation.

To improve the efficiency of androgenesis *in vitro*, it is necessary to conduct a comprehensive study of the structural features and cellular mechanisms of the reprogramming processes of anther cells from gametophytic to sporophytic pathway and their responsiveness to cultivation *in vitro*, and this technology still requires the development of a universal nutrient medium for all rice genotypes.

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References

1. Morinaga T., Fukushima E. Preliminary report on the haploid plant of rice, *Oryza sativa* L. // Proc. Imp. Acad. (Tokyo). – 1931. – No 7. – P. 383-384. https://doi.org/10.2183/pjab1912.7.383

2. Niizeki H, Oono K. Induction of haploid rice plant from anther culture // Proc Jpn Acad. – 1968. Vol. 44. – P. 554–557. https://doi.org/10.2183/pjab1945.44.554

3. Lantos C., Jancso M., Szekely A., Szaloki T., Venkatanagappa S., Pauk J. Development of in vitro anther culture for doubled haploid plant production in Indica Rice (*Oryza sativa* L.) Genotypes // Plants (Basel). – 2023. – Vol. 12, No 9. https://doi.10.3390/plants12091774

4. Kyum M., Kaur H., Kamboj A., Goyal L., Bhatia D. Strategies and prospects of haploid induction in rice (*Oryza sativa*) // Plant Breeding. – 2022. – Vol. 141, No 1. – P. 1-11. https://doi.org/10.1111/pbr.12971

5. Tripathy S.K., Lenka D., Prusti A.M., Mishra D., Swain D., Behera S.K. Anther culture in rice: Progress and breeding perspective // Applied Biological Research. – 2019. – Vol. 21, No 2. – P. 87-104. https://doi:10.5958/0974-4517.2019.00012.0

6. Patra S., Mohanty P., Tripathy S.K. Scope of androgenesis for genetic improvement in rice // Emergent Life Sciences Research. – 2019. – No 5. P. 23-28. https://doi.org/10.31783/elsr.2019.512328

7. Tripathy S.K. Anther Culture for Double Haploid Breeding in Rice-a Way forward // Rice Genomics and Genetics. – 2018. – Vol. 9, No 1. – P. 1-6. https://doi.10.5376/rgg.2018.09.0001

8. Mishra R., Rao G.J.N. In-vitro androgenesis in rice: advantages, constraints and future prospects // Rice Science. – 2016. – Vol. 23, No 2. – P. 57-68. https://doi.10.1016/j.rsci.2016.02.001

9. Huang C.S., Tsay H.S., Chern C.G., Chen, C.C. Japonica rice breeding using anther culture // Journal of Agriculture Research China. – 1988. – Vol. 37, No 1. – P. 1-8. https://scholars.tari.gov.tw/bitstream/123456789/13524/1/journal_arc_37-1-1.pdf

10. Bhojwani S.S., Pande H., Raina A. Factors Affecting Androgenesis in Indica Rice / Delhi, India: Department of Botany, University of Delhi. – 2001. http://geb.unigiessen.de/geb/volltexte/2003/1238/pdf/FestschriftNeumann_06.pdf

11. Ruwani D.M., Mayakudua G., Silva, T.D. Anther culture as a supplementary tool for rice breeding / In: Rice Crop – Current Developments. – 2018. [doi:10.5772/intechopen.76157], 1-15. https://www.intechopen.com/chapters/60519

12. Mayakaduwa D.M., Silva T.D. A cytological indicator allows rapid assessment of microspore maturity, leading to improved in vitro anther response in Indica rice (*Oryza sativa* L.) // In Vitro Cellular & Developmental Biology: Plant. – 2017. – Vol. 53, No 6. – P. 591-597. DOI: 10.1007/s11627-017-9855-0. https://doi.10.1007/s11627-017-9855-0

13. Yan J., Xue Q., Zhu J. Genetic studies of anther culture ability in rice (*Oryza sativa*) // Plant Cell, Tissue and Organ Culture. – 1996. – Vol 45. – P. 253-258.

14. Herath H.M.I, Bandara D.C., Samarajeewa P.K., Wijesundara D.S.A. Effect of low temperature pre-treatment on anther culture in selected indica, japonica rice varieties and their inter sub specific hybrids // Ceylon Journal of Science (Biological Sciences). – 2009. – Vol. 38, No 1. – P. 11-26.

15. Foroughi-Wehr B., Wenzel G. Andro- and parthenogenesis / In: Hayward MD, Bosemark NO, Romagosa I, Cerezo M, editors. Plant Breeding-Principles and Prospects. Dordrecht: Springer. – 1993. – P. 261-277.

16. Raina S.K., Zapata F.J. Enhanced anther culture efficiency of indica rice (Oryza sativa L.) through modification of the culture media // Plant Breed. – 1997. – Vol. 116, No 4. – P. 305-315. https://doi.10.1111/j.1439-0523.1997.tb01004.x

17. Niroula R.K., Bimb H.P. Effect of genotype and callus induction medium on green plant regeneration from anther of Nepalese rice cultivars // Asian Journal of Plant Sciences. – 2009. – No 8. – P. 368-374.

18. Germana M.A. Anther culture for haploid and doubled haploid production // Plant Cell, Tissue and Organ Culture. – 2011. – Vol. 104. – P. 283-300. https://doi.org/10.1007/s11240-010-9852-z

19. Murashige T., Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures // Physiologia Plantarum. – 1962. – Vol. 15, No 3. – P. 473–497. https://doi.10.1111/j.1399-3054.1962.tb08052

20. Gamborg O.L., Miller R.A., Ojima K. Nutrient requirements of suspension cultures of soybean root cells // Experimental Cell Research. – 1968. – Vol. 50, No 1. – P. 151–158. https://doi.10.1016/0014-4827(68)90403-5

21. Chu C.C. The N6 medium and its applications to anther culture of cereal crops / In Proceedings of Symposium on Plant Tissue Culture. Science Press. – 1978. – P. 43–50

22. Lentini Z., Reyes P., Martinez C.P., Roca W.M. Androgenesis of highly recalcitrant rice genotypes with maltose and silver nitrate // Plant Science – 1995. – Vol. 110. – P. 127–138. https://doi.org/10.1016/0168-9452(95)04180

23. Gill R., Kaur N., Sindhu A.S., Bharaj T.S., Gosal S.S. Improved methods for anther and pollen culture in rice / Advances in Rice Genetics. Philippines: International Rice Research Institute. – 2000. – P. 503-505.

24. Matsushima T., Kikuchi S., Takaiwa F., Oono K. Regeneration of plants by pollen culture in rice (*Oryza sativa* L.) // Plant Tissue Culture. – 1988. – Vol. 5. – P. 78–81. https://doi.10.5511/plantbiotechnology1984.5.78

25. Zapata-Arias F.J. Laboratory protocol for anther culture technique in rice / In: Maluszynski M., Kasha K.J., Forster B.P., Szarejko I., editors. Doubled Haploid Production in Crop Plants, a Manual. Dordrecht: Kluwer Academic Publishers. – 2003. – P. 109–116. https://doi.10.1007/978-94-017-1293-4_17

26. Gupta H.S., Borthakur D.N. Improved rate of callus induction from rice anther culture following microscopic staging of microspores in iron alum-haematoxylin // Theoretical and Applied Genetics. – 1987. – Vol 74. – P. 95–99. https://doi.org/10.1007/BF00290090

27. Lee S.T., Huang W.L. Osmotic stress stimulates shoot organogenesis in callus of rice (*Oryza sativa* L.) via auxin signaling and carbohydrate metabolism regulation // Plant Growth Regulation. – 2014. – Vol. 73. – P. 193–204. https://doi.org/10.1007/s10725-013-9880-x

28. Raina S.K., Irfan S.T. High frequency embryogenesis and plantlet regeneration from isolated microspores of indica rice // Plant Cell Reports. – 1998. – Vol. 17. – P. 957–962. https://doi.org/10.1007/s002990050517

29. Shariatpanahi M.E., Bal U., Heberle-Borsa, E., Touraev A. Stresses applied for the re-programming of plant microspores towards in vitro embryogenesis // Physiologia Plantarum. – 2006. – Vol. 127. – P. 519–534.

30. Chen Q.F., Wang C.L., Lu Y.M., Shen M., Afza R., Duren M.V., Brunner H. Anther culture in connection with induced mutations for rice improvement // Euphytica. – 2001. – Vol.120, No 3. – P. 401–408. https://doi.org/10.1023/A:1017518702176

31. Mkuya M.S., Hua-min S., Wen-zhen L., Zong-xiu S. Effect of 137Cs gamma rays to panicles on rice anther culture. Rice Science. – 2005. – Vol. 12, No 4. – P. 299–302. http://www.ricesci.org/CN/Y2005/V12/I4/299

32. Aldemita R.R., Zapata F.J. Anther culture of rice: effects of radiation and media components on callus induction and plant regeneration // Cereal Research Communications. – 1991. – Vol. 19, No 1/2. – P. 9–32. http://www.jstor.org/stable/23783734.

33. Nakamura K., Hattori K. Effect of 60Co gamma-ray irradiation at different culture stages on rice anther culture // Breeding Sci. – 1997. – Vol. 47, No 2. – P. 101–105. https://doi.org/10.1270/jsbbs1951.47.101

References

1. Aldemita, R.R., Zapata, F.J. (1991). Anther culture of rice: effects of radiation and media components on callus induction and plant regeneration. Cereal Research Communications 19 (1/2): 9–32. http://www.jstor.org/stable/23783734.

2. Bhojwani, S.S., Pande, H., Raina, A. (2001). Factors Affecting Androgenesis in Indica Rice / Delhi, India: Department of Botany, University of Delhi. http://geb.unigiessen.de/geb/volltexte/2003/1238/pdf/FestschriftNeumann_06.pdf

3. Chen, Q.F., Wang, C.L., Lu, Y.M., Shen, M., Afza, R., Duren, M.V., Brunner, H. (2001). Anther culture in connection with induced mutations for rice improvement / Euphytica. 120(3), 401-408. https://doi.org/10.1023/A:1017518702176

4. Chu, C.C. (1978) The N6 medium and its applications to anther culture of cereal crops. In Proceedings of Symposium on Plant Tissue Culture. Science Press, 43-50

5. Foroughi-Wehr, B., Wenzel, G. (1993). Andro- and parthenogenesis. In: Hayward MD, Bosemark NO, Romagosa I, Cerezo M, editors. Plant Breeding-Principles and Prospects. Dordrecht: Springer, 261-277.

6. Gamborg, O.L., Miller, R.A., Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research. 50 (1): 151–158. https://doi.10.1016/0014-4827(68)90403-5

7. Germana, M.A. (2011). Anther culture for haploid and doubled haploid production. Plant Cell, Tissue and Organ Culture, 104, 283-300. https://doi.org/10.1007/s11240-010-9852-z

8. Gill, R., Kaur, N., Sindhu, A.S., Bharaj, T.S., Gosal, S.S. (2000). Improved methods for anther and pollen culture in rice. Advances in Rice Genetics. Philippines: International Rice Research Institute, 503-505.

9. Gupta, H.S., Borthakur, D.N. (1987). Improved rate of callus induction from rice anther culture following microscopic staging of microspores in iron alum-haematoxylin. Theoretical and Applied Genetics. 74:95-99. https://doi.org/10.1007/BF00290090

10. Herath, H.M.I, Bandara, D.C., Samarajeewa, P.K., Wijesundara, D.S.A. (2009) Effect of low temperature pre-treatment on anther culture in selected indica, japonica rice varieties and their inter sub specific hybrids. Ceylon Journal of Science (Biological Sciences), 38(1): 11-26.

11. Huang, C.S., Tsay, H.S., Chern, C.G., Chen, C.C. (1988). Japonica rice breeding using anther culture. Journal of Agriculture Research China. 37(1), 1-8. https://scholars.tari.gov.tw/bitstream/123456789/13524/1/journal_arc_37-1-1.pdf

12. Kyum, M., Kaur, H., Kamboj, A., Goyal, L., Bhatia, D. (2022). Strategies and prospects of haploid induction in rice (Oryza sativa). Plant Breeding, 141(1), 1-11. https://doi.org/10.1111/pbr.12971

13. Lantos, C., Jancso, M., Szekely, A., Szaloki, T., Venkatanagappa, S., Pauk, J. (2023). Development of in vitro anther culture for doubled haploid plant production in Indica Rice (Oryza sativa L.). Genotypes. Plants (Basel), 12(9). https://doi.10.3390/plants12091774

14. Lee, S.T., Huang, W.L. (2014). Osmotic stress stimulates shoot organogenesis in callus of rice (Oryza sativa L.) via auxin signaling and carbohydrate metabolism regulation. Plant Growth Regulation. 73, 193-204. https://doi.org/10.1007/s10725-013-9880-x

15. Lentini, Z., Reyes, P., Martinez, C.P., Roca, W.M. (1995). Androgenesis of highly recalcitrant rice genotypes with maltose and silver nitrate. Plant Science, 110, 127-138. https://doi.org/10.1016/0168-9452(95)04180

16. Matsushima, T., Kikuchi, S., Takaiwa, F., Oono, K. (1988) Regeneration of plants by pollen culture in rice (Oryza sativa L.). Plant Tissue Culture. 5:78-81. https://doi.10.5511/plantbiotechnology1984.5.78

17. Mayakaduwa, D.M., Silva, T.D. (2017). A cytological indicator allows rapid assessment of microspore maturity, leading to improved in vitro anther response in Indica rice (Oryza sativa L.). In Vitro Cellular & Developmental Biology: Plant. 53(6), 591-597. DOI: 10.1007/s11627-017-9855-0. https://doi.10.1007/s11627-017-9855-0

18. Mishra, R., Rao, G.J.N. (2016). In-vitro androgenesis in rice: advantages, constraints and future prospects, Rice Science, 23(2), 57-68. https://doi.10.1016/j.rsci.2016.02.001

19. Mkuya, M.S., Hua-min, S., Wen-zhen, L., Zong-xiu, S. (2005). Effect of 137Cs gamma rays to panicles on rice anther culture. Rice Science. 12(4), 299-302. http://www.ricesci.org/CN/Y2005/V12/I4/299

20. Morinaga, T., Fukushima, E. (1931). Preliminary report on the haploid plant of rice, *Oryza sativa* L. Proc. Imp. Acad. (Tokyo), 383-384. https://doi.org/10.2183/pjab1912.7.383

21. Murashige, T., Skoog F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum, 15 (3): 473–497. https://doi.10.1111/j.1399-3054.1962.tb08052

22. Nakamura, K., Hattori, K. 1997. Effect of 60Co gamma-ray irradiation at different culture stages on rice anther culture. Breeding Sci 47(2): 101-105. https://doi.org/10.1270/jsbbs1951.47.101

23. Niizeki H, Oono K. (1968). Induction of haploid rice plant from anther culture. Proc Jpn Acad. 44, 554–557. https://doi.org/10.2183/pjab1945.44.554

24. Niroula, R.K., Bimb, H.P. (2009). Effect of genotype and callus induction medium on green plant regeneration from anther of Nepalese rice cultivars. Asian Journal of Plant Sciences, 8, 368-374.

25. Patra, S., Mohanty, P., Tripathy, S.K. (2019). Scope of androgenesis for genetic improvement in rice. Emergent Life Sciences Research, 5, 23-28. https://doi.org/10.31783/elsr.2019.512328

26. Raina, S.K., Irfan, S.T. (1998). High frequency embryogenesis and plantlet regeneration from isolated microspores of indica rice / Plant Cell Reports. 17, 957-962. https://doi.org/10.1007/s002990050517

27. Raina, S.K., Zapata, F.J. (1997). Enhanced anther culture efficiency of indica rice (Oryza sativa L.) through modification of the culture media. Plant Breed, 116(4), 305-315. https://doi.10.1111/j.1439-0523.1997.tb01004.x

28. Ruwani, D.M., Mayakudua, G., Silva, T.D. (2018). Anther culture as a supplementary tool for rice breeding. In: Rice Crop – Current Developments. [doi:10.5772/intechopen.76157], 1-15. https://www.intechopen.com/chapters/60519

29. Shariatpanahi, M.E., Bal, U., Heberle-Borsa, E., Touraeva, A. (2006). Stresses applied for the re-programming of plant microspores towards in vitro embryogenesis. Physiologia Plantarum. 127, 519-534.

30. Tripathy, S.K. (2018) Anther Culture for Double Haploid Breeding in Rice-a Way forward. Rice Genomics and Genetics 9(1): 1-6. https://doi.10.5376/rgg.2018.09.0001

31. Tripathy, S.K., Lenka, D., Prusti, A.M., Mishra, D., Swain, D., Behera, S.K. (2019). Anther culture in rice: Progress and breeding perspective / Applied Biological Research. 21(2), 87-104. https://doi:10.5958/0974-4517.2019.00012.0

32. Yan, J., Xue, Q., Zhu, J. (1996). Genetic studies of anther culture ability in rice (*Oryza sativa*). Plant Cell, Tissue and Organ Culture, 45, 253-258.

33. Zapata-Arias, F.J. (2003). Laboratory protocol for anther culture technique in rice. In: Maluszynski M., Kasha K.J., Forster B.P., Szarejko I., editors. Doubled Haploid Production in Crop Plants, a Manual. Dordrecht: Kluwer Academic Publishers; 109-116. https://doi.10.1007/978-94-017-1293-4_17

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