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Optimization of *In-vitro* Androgenesis Protocol for the Efficient Production of Haploids and Doubled Haploids in Diverse Rice Cultivars

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Abstract

In-vitro androgenesis is a powerful biotechnological tool that enables rapid conversion of heterozygous hybrids into fully homozygous lines, significantly accelerating the breeding process. Despite its potential, its application in *indica* rice hybrids has been limited due to poor regenerative calli formation, low regeneration rates and frequent production of albino plants. This study therefore aimed to optimize callus induction and regeneration protocols for improved efficiency in *indica* rice hybrids, *japonica* cultivars and the derivatives of *indica* × *japonica* cross. Anthers found in the spikelets positioned at 2/3rd position on the panicle were cultured on five different callus induction media (C1-C5). Among these, the C1 medium comprising N6 basal media supplemented with 2 mg/L 2,4-D; 1 mg/L NAA; 0.5 mg/L kinetin; 3% maltose and 0.2% clerigel, with a pH of 5.8 and previously optimized for the *indica* rice hybrid, KRH 4 produced the highest callus induction efficiency. This medium also yielded a higher percentage of regenerative calli (25.14% for PRH 56). Upon culturing of these regenerative calli on 5 different regeneration media, shoot regeneration was observed only in the S5 regeneration medium (MS basal salts) with 0.5 mg/L NAA; 1 mg/L BAP; 3 mg/L kinetin; 3% sucrose and 0.7% agar. This study also emphasizes the critical role of the micro-environment in obtaining healthy regenerants. Plantlets grown in culture tubes exhibited better health and vigor compared to those developed in culture bottles. The optimized protocol was particularly effective in producing robust haploid regenerants. Evaluation of spontaneous chromosome doubling revealed that, *japonica* cultivars produced a significantly higher proportion of doubled haploids (92.92%) compared to *indica* cultivars (8.79%). In contrast, 91% of the regenerants from the *indica* type were haploids, indicating the need for artificial chromosome doubling. These findings provide valuable insights for improving androgenic response and doubled haploid (DH) production efficiency in diverse rice backgrounds.

Keywords : Anther culture, Haploids, Doubled haploids (DH), Genotype response

Globally, rice is one of the major cereal crops consumed as a staple food for more than half of the world's population and is the third largest grain produced in the world. By 2050, the global population is projected to reach 10.6 billion, leading to an increased demand for rice (Valera and Pede, 2023). With increasing demands due to population growth and the challenges posed by climate change, there is a pressing need to accelerate rice breeding for improved varieties. One promising approach to achieve this is through doubled haploid (DH) technology, which enables the rapid development of completely homozygous lines in a single generation (Sanasam *et al*., 2016, Iqbal & Yousaf, 2018, Samantaray *et al* 2021, Ghalagi & Raju, 2022, Kotyal., 2022, Lantos *et al*., 2022 and Islam *et al*., 2023). Doubled haploids (DHs) can also be used in genetic mapping of complex qualitative traits (Pooja & Sheshshayee, 2017), facilitating linkage analysis and recombination fraction estimation. They also help in revealing recessive mutations, eliminating transgenic hemizygotes and enabling reverse breeding strategies (Dunwell, 2010 and Dwivedi *et al*., 2015). Further, doubled haploid lines offer genetic uniformity within the lines and trait variation among the lines, enabling efficient selection of superior rice cultivars for varietal release (Sanasam *et al*., 2019). Despite significant progress in rice anther culture, challenges such as low regeneration frequency, albinism, poor hardening success and post-acclimatization mortality still hinder the widespread application of anther culture in breeding programs (Mishra *et al*., 2016).

Higher anther culture efficiency and larger progeny populations are crucial for the success of anther culture in rice breeding programs (Li *et al.,* 2015). Studies suggests that, japonica rice cultivars or hybrids are more responsive to anther culture than indica rice types, emphasising the genotype specificity of rice androgenesis (Grewal *et al*., 2011). Therefore, in the present study, three diverse rice types were chosen to optimise the anther culture protocol on callusing and regeneration efficiency in rice (Table 3). Additionally, two different culture conditions were compared for their influence on seedling vigour, seedling survival and eventual growth.

In this study, we aim to contribute towards the refinement of rice anther culture protocols by integrating optimized media and culture conditions, thereby facilitating the development of robust DH lines suitable for use in breeding programmes.

Material and Methods

Plant Material and Cultivars

Genotype is the most important factor affecting the efficiency of anther culture in rice (Sarao and Gosal., 2018). In addition to genotype, other factors such as the developmental stage of microspores, pre-treatment conditions, culture media composition and physical conditions during tissue culture also play significant roles in determining success (Mayakaduwa and Silva, 2023). Therefore, it is essential to establish a standardized, efficient and high-through put protocol for the large-scale production of haploids through anther culture. In this context, a study was undertaken to develop a reliable and reproducible protocol for anther culture and for generation of androgenic plants in diverse rice cultivars such as PRH 56, KRH 12 (Indica type), Nipponbare and Taipee (Japonica type), MSN 111 and MSN 101 (Derivatives of indica X Japonica cross). The donor plants were grown under controlled greenhouse conditions to ensure uniform growth and optimal panicle development.

Collection and Pre-treatment of Panicles

Panicles at the booting stage were collected from the primary tillers of all the six rice cultivars between 07:00 and 08:00 am. The developmental stage was standardized for each genotype by measuring the distance between the base of the flag leaf and the node of the boot leaf, followed by microscopic examination to confirm the presence of more uninucleate microspores (Mayakaduwa *et al*., 2017) using 1 per cent acetocarmine dye (Islam *et al*., 2013). Based on this assessment, panicles were harvested at genotype-specific lengths corresponding to the desired developmental stage. Surface sterilized panicles were kept for cold pre-treatment at 10°C for 7 days (Dalpat *et al*., 2014, Kotyal *et al*., 2022 and Wang *et al*., 2022). Cold treated panicles were sterilized by using 70 per cent ethanol for 1 minute followed by 0.1 per cent of $HgCl_2$ for 3 min and washed thoroughly with sterile water (Rukmini *et al*., 2013).

Anther Isolation and Inoculation

Under aseptic conditions, anthers found in spikelets at 2/3rd position of the panicle where the microspores at uninucleate stage were carefully dissected (Ghalagi and Raju, 2022) and inoculated onto callus induction medium (CIM). Five CIM (C1to C5) were tried with N6 (Chu *et al*., 1975) as the basal medium (Table 1). The inoculated anthers were

Table 1
N6 media supplemented various PGRs for callus induction in different rice cultivars

Treatments	Carbon source (%)		Plant growth regulators supplemented (mg/l)				Gelling agent (%)	
	Maltose	Sucrose	2,4-D	NAA	Kn	BAP	Clerigel	Agar
C1	3.0	-	2.0	1.0	0.5	-	0.2	-
C2	-	3.0	2.0	1.0	0.5	-	0.2	-
C3	3.0	-	2.0	1.5	0.5	-	-	0.8
C4	3.0	-	2.0	-	-	-	0.2	-
C5	3.0	-	2.0	1.0	-	0.5	0.2	-

incubated in dark at 25 ± 2 °C and the observation on the anther response to callus induction was recorded 3-4 weeks after inoculation (Kaushal *et al.,* 2014, Debina *et al.,* 2016 and Ghalagi & Raju, 2022)

Shoot and Root Regeneration

Regenerative calli measuring 1-2 mm were used for green shoot regeneration using five different media formulations, designated as S1 to S5 (Table 2). Cultures were maintained under 16-hour light / 8-hour dark regime at 28 ± 2°C with an artificial light (2000 lux) and monitored for shoot regeneration. The regenerated shoots were subsequently transferred to MS medium supplemented with 1mg/l NAA; 0.1 mg/l Kinetin and 5 per cent sucrose after adjusting the pH to 5.8 for root induction (Table 2). Regeneration frequency and morphological traits were recorded after the development of regenerative calli.

Regenerated plantlets were maintained in two types of culture vessels; culture tubes and culture bottles. The difference in plant growth and vigour was recorded between the two methods. Further, the androgenic plants were identified and grouped them as Haploids and Doubled Haploids based on the leaf tip characteristics as reported by Ghalagi, *et al.,* (2023). The efficiency of diploidization through spontaneous chromosome doubling in different cultivars were analysed by this method.

Statistical Analysis

The data generated were analysed using GraphPad Prism 8.0.1 244 and DMRT was performed using

Table 2
Basal media supplemented with different PGRs for shoot and root induction in different rice cultivars

Organogenesis	Treatments	Basal media	Plant growth regulators supplemented (mg/l)				Gelling agent (%)	
			Sucrose	NAA	Kn	BAP	Clerigel	Agar
Shoot	S1	N6	3.0	0.5	1.0	3.0	0.2	-
	S2	N6	3.0	-	2.0	0.5	0.2	-
	S3	N6	3.0	0.2	2.5	1.0	-	0.6
	S4	N6	3.0	0.5	2.0	1.0	0.2	-
	S5	MS	3.0	0.5	3.0	1.0	-	0.7
Root	R1	MS	5.0	1.0	0.1	-	-	0.7

OPSTAT. Each experiment was conducted with 10 replications each

Results and Discussion

Refined Callus Induction Strategy to Improve Callus Formation Efficiency

Anther culture being highly genotype dependent, efforts were made to enhance the efficiency of anther culture in a genotype-independent manner. To overcome these limitations and to improve the efficiency of anther culture across cultivars, increased focus was given on optimizing external factors such as culture media composition and pre and post-culture conditions. The stage of anther containing microspores play a major role here. Hence, precise selection of anthers at uninucleate stage is very important for improving callus induction rates in rice (Zapata, 2003). Accordingly, we have standardized the distance between the boot and flag leaf for each cultivar to determine the stage at which the highest number of uninucleate microspores are present. In the indica rice hybrids, KRH 12 and PRH 56, the optimal distances were 12 cm and 14 cm, respectively. Similarly, for the japonica varieties, Nipponbare and Taipee, the ideal distance was 10 cm, while for the derivatives of indica x japonica cross, MSN 111 and MSN 101, the corresponding distances were 8 cm and 6 cm, respectively (Table 3). In the previous study by Debina *et al.*, (2016), it was reported that, for KRH 4 rice hybrid, anthers obtained from panicles of 8-12 cm from flag leaf to penultimate leaf showed

Table 3

Standardized boot-flag leaf length for efficient callusing in different cultivars

Rice type	Rice cultivars	Boot-flag leaf length (cm)
Indica	KRH 12	12
	PRH 56	14
Japonica	Nipponbare	10
	Taipee	10
Indica x Japonica	MSN 111	8
	MSN 101	6

efficient culturability. Besides the boot-flag length, the positioning of the spikelets also regulates the callusing ability (Afza *et al.*, 2000). Recently, Ghalagi and Raju, (2023) have reported that, the spikelets from the middle portion of the panicle was found to be very effective in callus induction and therefore, in our study, anthers found inside the spikelets positioned at 2/3rd position of the panicle were inoculated.

Callus induction efficiency (%) among different cultivars was found to be significantly higher in C1 media compared to the others with PRH 56 producing 54.31% followed by Nipponbare producing 46.02% (Fig. 1). This media (C1) has basal N6 media supplemented with 2, 4-D (2mg/l), NAA (1mg/l), Kinetin (0.5mg/l), Maltose (3%) and Clerigel (0.2%). Although the C2 medium had the same composition

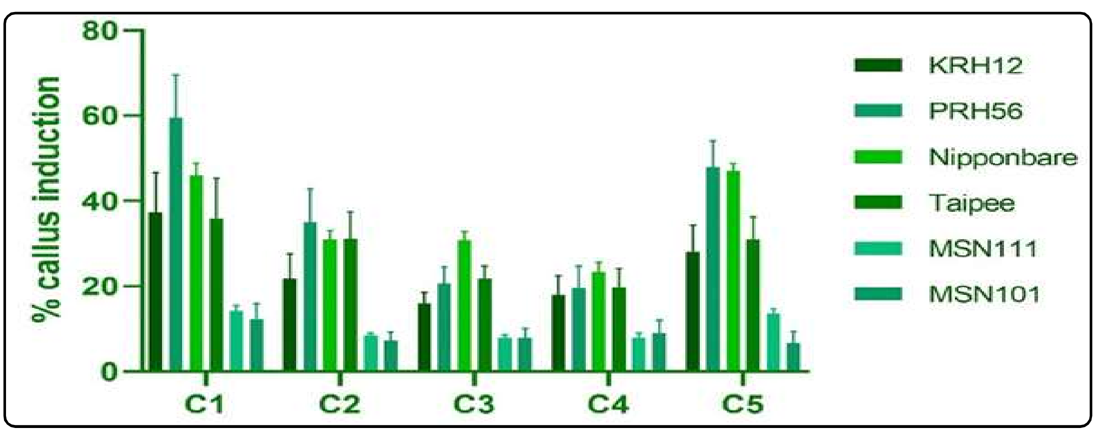

Fig. 1 : Callus induction in different rice cultivars in different media

as C1, with sucrose substituted for maltose, it resulted in lower callus induction efficiencies with only 35.16% in PRH 56 and 30.94% in Nipponbare. As noticed by Mostafiz and Wagiran (2018), maltose seems to be a more effective carbohydrate source than sucrose for promoting androgenesis across various species, including cereals. Sen and Singh (2011) and Rukmini *et al*, (2013) have also highlighted the superiority of maltose over sucrose, attributing its advantage to slower decomposition in the culture medium. This slow breakdown allows maltose to function as a stable osmoticum, thereby helping maintain medium stability and preventing microspore plasmolysis. The C5 medium, which differed from C1 only by the use of BAP instead of kinetin, yielded comparable results with PRH 56 achieving 47.88% and Nipponbare 46.97% callusing efficiency. Among the tested cultivars, *indica* rice hybrids, PRH 56 and KRH 12 produced higher percentage of regenerative calli (22.45% and 25.14%, respectively) compared to *japonica* cultivars, Nipponbare and Taipei (20.7% and 19.76% respectively) (Fig. 2). However, the derivatives of indica × japonica cross exhibited very low callusing and regenerative calli, indicating a higher level of recalcitrancy, suggesting the need for further refinement in media composition and stress treatments to improve their androgenic response.

To enhance shoot regeneration efficiency in *indica* rice hybrids, it is essential to optimize both the type and concentration of plant growth regulators in the regeneration medium. To evaluate regeneration efficiency, regenerative calli (1-2 mm) derived from C1 culture were transferred to different regeneration media (S1-S5). Plant regeneration occurred only in

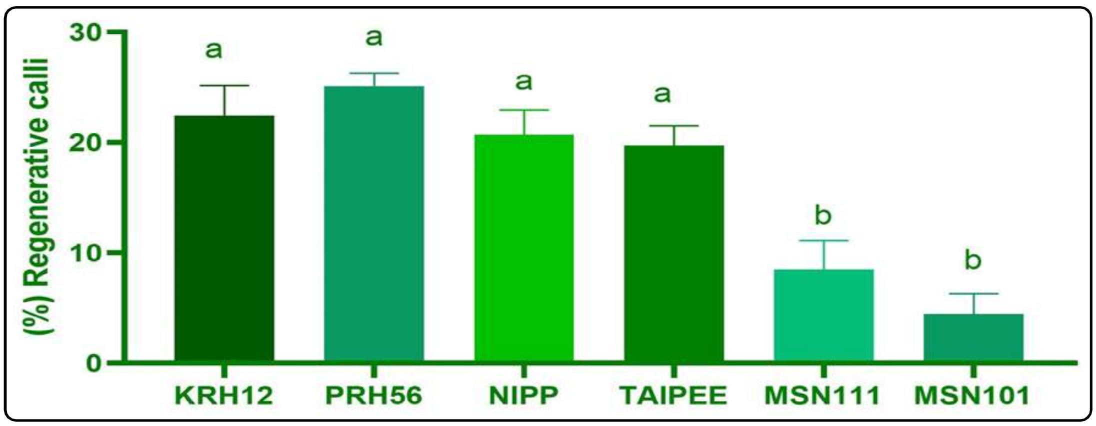

Fig. 2 : Regenerative calli from different rice cultivars

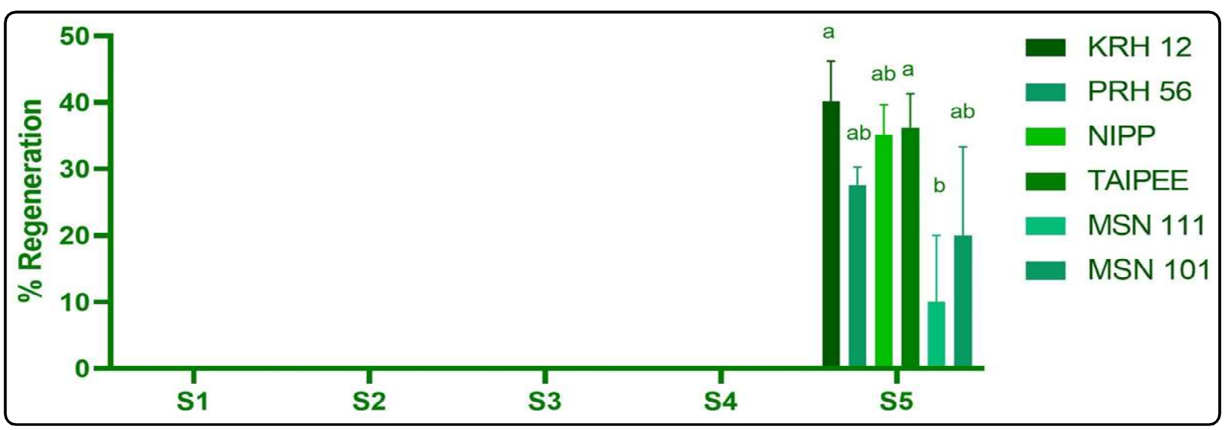

Fig. 3 : Regeneration media on shoot induction

S5 medium (Fig. 3), comprising MS basal salts supplemented with 3 mg/L kinetin, 1 mg/L BAP, 0.1 mg/L NAA, 3% sucrose and 0.7% agar at a pH of 5.8. Surprisingly, none of the other media induced the shoot regeneration in any of the rice cultivars used. It appears that, the N6 basal media used in all the other treatments (S1-S4) could be the reason for the failed regeneration in all the rice cultivars. Ali *et al.* (2021) however, through modifying the N6 medium by incorporating varying concentrations of 2,4-D, NAA and cytokinins, could show improved callus formation and plant regeneration in *japonica*, *indica* and their inter sub-specific hybrids. In general, N6 medium, originally formulated by Chu *et al*. (1975), was specifically developed for callus induction in rice anthers and may lack sufficient nutrients required for embryo development and subsequent morphogenesis (Appendix 1). Murashige and Skoog (MS) medium on the other hand provides a more supportive environment for plant regeneration due to its high total nitrogen content, with both nitrate (NO) and ammonium (NH_4^+) forms present in abundance and a relatively higher ammonium-to-nitrate ratio (Phillips and Garda, 2019). The NO_3^- : NH_4^+ ratio has been identified as a crucial determinant for the success of anther culture and the *in-vitro* induction of regenerative calli, particularly in *indica* rice (Grimes and Hodges, 1990). Furthermore, the absence of myo-inositol in N6 medium may impair cell wall formation and membrane biosynthesis, both essential for shoot regeneration (Loewus and Murthy, 2000). Additionally, critical micronutrients such as zinc (Zn), manganese (Mn), copper (Cu) and cobalt (Co) which are required for chlorophyll biosynthesis and organogenic gene expression are either deficient or absent in N6 medium.

With respect to regeneration efficiency of the regenerative calli cultured on S-5 medium, it was almost similar in both indica and japonica types with very poor regeneration in the derivatives of indica x japonica cross (Fig. 4). Based on our observations, the low callus induction and regeneration efficiency observed in the derivatives of *indica* x *japonica* cross may be attributed to their smaller anther size with a fewer uninucleate microspores, which probably reduce their regeneration success (unpublished)

Since the rooting has not occurred in those regenerated plantlets, the regenerated green shoots were transferred to MS medium supplemented with NAA (1.0 mg/L), kinetin (0.1 mg/L), 50 g/L sucrose and 0.7% agar for root development, where the 10:1 ratio of auxin to cytokinin successfully induced 100% rooting across all the cultivars. These findings are consistent with Pattnaik *et al*, (2020), who reported effective rooting using NAA (2.0 mg/L) + kinetin (0.5 mg/L) with 50 g/L sucrose.

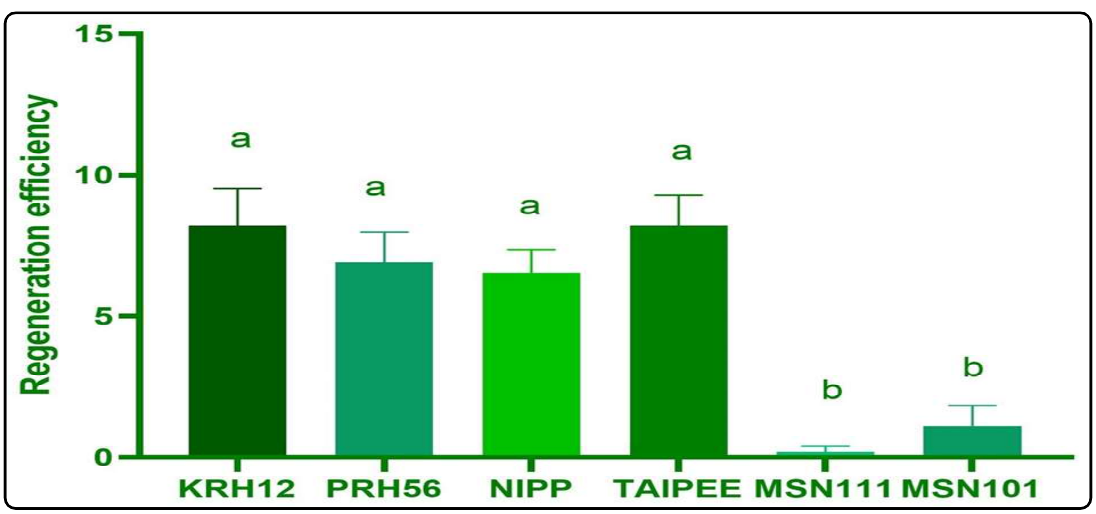

Fig. 4 : Regenerative efficiency in different cultivars

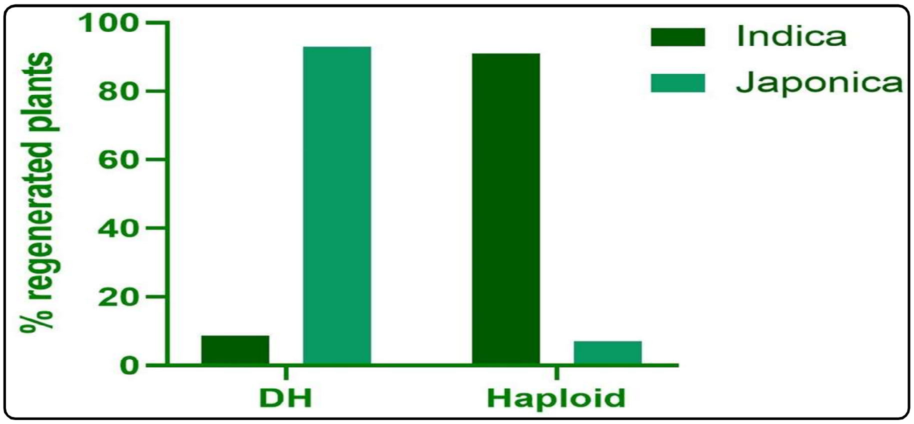

Fig. 5 : Regenerated plants from *indica* and *japonica* cultivars. (n=216 in *indica* and n = in *japonica*

Although a good number of regenerants were obtained from both indica and japonica backgrounds, evaluation of spontaneous chromosome doubling revealed that, japonica cultivars produced a significantly higher proportion of doubled haploids (92.92%) compared to indica cultivars (8.79%). In contrast, 91% of the regenerants from the indica hybrid were haploids (Fig. 5), underscoring the need for artificial chromosome doubling in these cultivars. These regenerated plants were categorized as haploids and DHs based on their leaf tip morphology and tallness of the plants. Those plants that are taller and with pointed (attenuate) leaf tips were identified as doubled haploids, while shorter plants with blunt (acute) leaf tips were classified as haploids (Ghalagi *et al.,* 2023). Since we did not use any markers in our study to distinguish diploids from doubled haploids (DHs), the presence of diploids (generated from anther wall during androgenesis) cannot be ruled out. However, research carried out in our laboratory on androgenesis of rice for the past 10 years showed no diploid plants in any of the rice backgrounds used and therefore, we may assume that those plants with attenuate leaf shape (pointed leaf tip) are doubled haploids.

Effect of Micro-environment on Growth of Regenerants

Culture environment plays a major role on the growth and development of regenerants. The regenerated plants were transferred to culture tubes and culture bottles (Plate 1) and the growth of regenerants were evaluated. Plants incubated in culture tubes were healthier with straight, open leaves, and exhibited robust growth while those grown in culture bottles exhibited weak growth, pale and crinkled leaves and reduced vigour (Plate 2). Plants grown in culture tubes exhibited greater root development compared to those grown in culture bottles (Plate 3). Culture tube grown plants

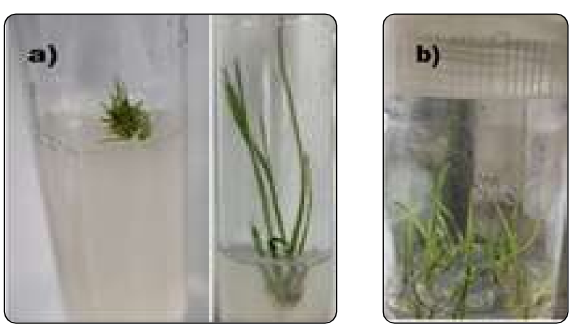

Plate 1 : Representative image of regenerants maintained in culture tube (a) and culture bottle (b)

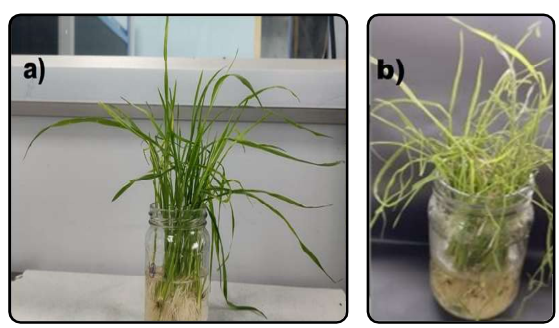

Plate 2 : Phenotypic appearance of plants cultured in tubes (a) and bottle (b) during hardening

Plate 3 : Representative image of plants grown in culture tubes (left) and culture bottles (right) post hardening

Plate 4 : Phenotype of culture tube (a) and culture bottle (b) grown plants in green house conditions. White arrow indicate dried crinkled leaf in the green house conditions

performed better in the greenhouse conditions (Plate 4a) while the culture bottle grown plants are not only weak but took longer time to acclimatize to greenhouse conditions (Plate 4b) and many failed to survive during the acclimatization phase (Fig. 6). Huang and Chen (2005) emphasized the importance of selecting culture vessels with optimal physical properties to enhance gas exchange, ensure uniform light distribution, and reduce stress caused by ethylene and CO_2 build up. In the present study, culture tubes likely provided more efficient gas exchange and better light distribution compared to culture bottles sealed with plastic lid which prevented proper gas exchange and light distribution.

The present study highlights the critical role of genotype, culture media composition and culture conditions in optimizing androgenesis and plant regeneration in rice. Although anther culture is inherently genotype-dependent, this limitation can

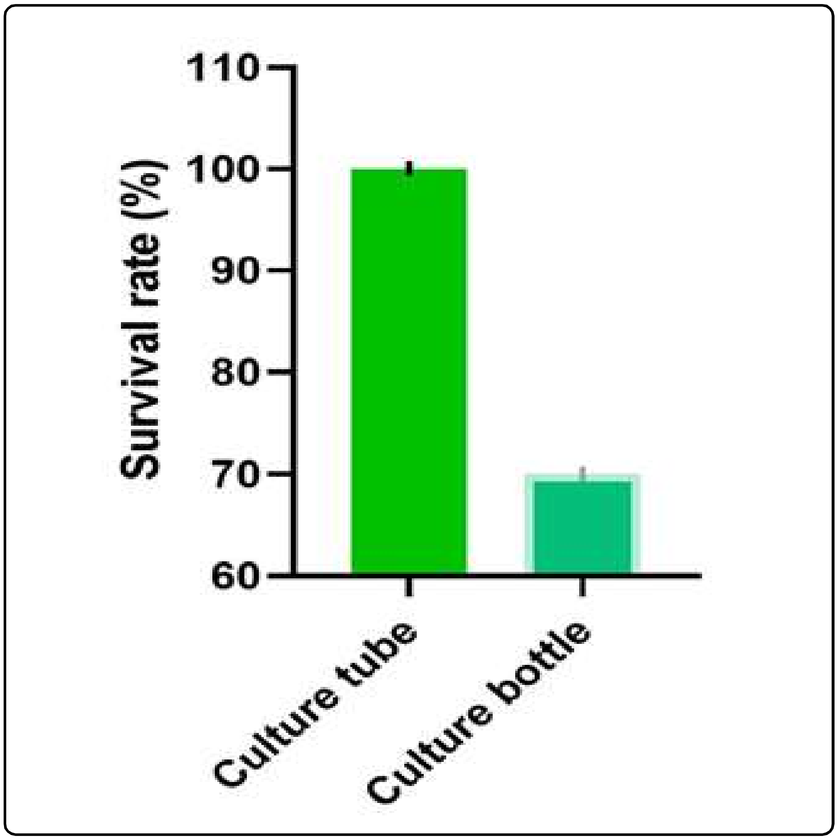

Fig. 6 : Survival rate of plants grown in culuture tubes and culture bottles in greenhouse conditions

be significantly mitigated by optimizing external factors such as the selection of responsive microspore stages, panicle positions, and precise media formulations. Among the tested culture media, the use of maltose over sucrose, as demonstrated in C1 medium, substantially enhanced the callus induction frequencies, confirming its efficacy as a stable osmoticum. Additionally, replacement of kinetin with BAP showed comparable results, indicating flexibility in cytokinin choice.

Importantly, regeneration was only successful on MS-based medium (S5), emphasizing the need for a nutritionally rich environment with balanced NO_3^- : NH_4^+ ratio, essential micronutrients, and myo-inositol for successful morphogenesis. MS medium not only supported regeneration but also facilitated 100% rooting when supplemented with an optimal auxin-to-cytokinin in 10:1 ratio. The micro environment also significantly influenced the growth and acclimatization of regenerants, with culture tubes proving more favorable than bottles. These findings underline the necessity of an integrated approach involving genotype screening, media optimization, and culture environment refinement to improve the efficiency of haploid and doubled haploid production in both indica and japonica rice backgrounds.

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References

Afza, R., Shen, M., Zapata-Arias, F. J., Xie, J., Fundi, H. K., Lee, K. S., Bobadilla-Mucino, E. and Kodym, A., 2000. Effect of spikelet position on rice anther culture efficiency. *Plant Science*, **153** (2) : 155 - 159.

Ali, J., Nicolas, K. L. C., Akther, S., Torabi, A., Ebadi, A. A., Marfori-Nazarea, C. M. and Mahender, A., 2021, Improved anther culture media for enhanced callus formation and plant regeneration in rice (*Oryza sativa* L.). *Plants*, **10** (5) : 839.

Chu, C. C.,Wang, C. C., Sun, C. S., Hsu, C., Yin, K. C., Chu, C. Y. and Bi, F. Y., 1975, Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Scientia Sinica,* **16** : 659 - 688.

Chu, C., Chu, W. C., San, S. C., Chen, H., Chu, Y. K. and Yin, C. C., 1975, Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources.

Dalpat, L., Shashidhar H. E., Gowda, P. H. R. and Ashok, T. H. 2014 Callus induction and regeneration from *In-vitro* anther culture of rice (*Oryza sativa* L.). *Int. J. Agrl. Envir. Biotechnol.,* **7** (2) : 213 - 218.

Debina, S., Udayakumar, M., Shivakumar, N., Sheshshayee, M. S. and Raju, B. M., 2016, Anther derived haploid production in rice and identification of true haploids by markers and flow cytometry *Mysore J. Agric. Sci*., **50** (2) : 305 - 308.

Dunwell, J.M., 2010. Haploids in flowering plants: origins and exploitation. *Plant biotechnology journal*, **8** (4) : 377 - 424.

Dwivedi, S. L., Britt, A. B., Tripathi, L., Sharma, S., Upadhyaya, H. D. and Ortiz, R., 2015, Haploids: constraints and opportunities in plant breeding. *Biotechnology advances*, **33** (6) : 812 - 829.

Ghalagi, C. and Raju, B. M., 2022, Optimization of *In-vitro* Androgenic Protocol for development of Haploids and Doubled Haploids in Indica Rice Hybrid, KRH 4. *Mysore J. Agric. Sci*., **56** (1).

Ghalagi, C., Namratha, M. R., Kotyal, K., Prakash, S. and Raju, B. M., 2023, A novel visual marker to distinguish haploids from doubled haploids in rice (*Oryza sativa*, L.) at early growth stages. *Plant Methods*, **19** (1) : 137.

Grewal, D., Manito, C. and Bartolome, V., 2011, Doubled haploids generated through anther culture from crosses of elite indica and japonica cultivars and lines of rice: Large scale production, agronomic performance and molecular characterization. *Crop Sci.,* **1** (6) : 2544 - 2553.

Grimes, H. D., Hodges, T. K., 1990, The inorganic NO_3^- : NH_4^+ ratio influences plant regeneration and auxin sensitivity in primary callus derived from immature embryos of indica rice (*Oryza sativa* L.). *J. Plant Physiol.,* **136** : 362 - 367.

Huang, C. and Chen, C., 2005, Physical properties of culture vessels for plant tissue culture. *Biosystems engineering*, **91**(4) : 501 - 511.

Iqbal, J. and Yousaf, U., 2018, Anther culturing a unique methodology in achieving homozygosity. *Asian J. Adv. Agric. Res.*, **8** (2) : 1 - 9.

Islam, M. T., Arif, M. R., Hasan, M. T. and Robin, A. H. K., 2023, Anther culture in crop plants: progress and perspectives.

Islam, S. S., Ara, I., Tuteja, N. and Subramaniam, S., 2013, Efficient microspore isolation methods for high yield embryoids and regeneration in rice (*Oryza sativa* L.). *World Aca. Sci, Engi. Tech. (WASET), Inter. J. Biol. Sci. Engi.*, **7** (12) : 891 - 96.

Kaushal, L., Balachandran, S. M., Ulaganathan, K. and Shenoy, V., 2014, Effect of culture media on improving anther culture response of rice (*Oryza sativa* L.). *International Journal of Agriculture Innovations and Research*, **3** (1) : 218 - 224.

Kotyal, K., Ghalagi, C., Namratha, M. R. and Mohan Raju, B., 2022, Pyramiding of drought adaptive traits and development of doubled haploids in the traits pyramided rice (*Oryza sativa* L.). *Plant Physiology Reports*, **27** (3) : 458 - 468.

Lantos, C., Jancsó, M., Székely, Á., Nagy, É., Szalóki, T. and Pauk, J., 2022, Improvement of anther culture to integrate doubled haploid technology in temperate rice (*Oryza sativa* L.) breeding. *Plants*, **11** (24) : 34 - 46.

Li, S., Chen, Z., Liu, K., Yang, G., Wang, F., Hu, G., Zhou, L., Zha, W., You, A., **2015**, Study on anther culture condition of indica rice male sterile line. *Hubei Agric. Sci.* **54** : 4868 - 4870.

Loewus, F. A. and Murthy, P. P., 2000, myo-Inositol metabolism in plants. *Plant science*, **150** (1) : 1 - 19.

Mayakaduwa, D. M. R. G. and 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** : 591 - 597.

Mayakaduwa, R. and Silva, T., 2023, Haploid induction in indica rice: exploring new opportunities. *Plants*, **12** (17) : 3118.

Mishra, R. and Rao, G. J. N., 2016, *In-vitro* and rogenesis in rice: advantages, constraints and future prospects. *Rice Science*, **23** (2) : 57 - 68.

Mostafiz, S. and Wagiran, A., 2018, Efficient callus induction and regeneration in selected indica rice. *Agronomy*, **8** (5) : 77.

Pattnaik, S.S., Dash, B., Bhuyan, S. S., Katara, J. L., Parameswaran, C., Verma, R., Ramesh, N. and Samantaray, S., 2020, Anther culture efficiency in quality hybrid rice: A comparison between hybrid rice and its ratooned plants. *Plants*, **9** (10) : 1306.

Phillips, G. C. and Garda, M., 2019, Plant tissue culture media and practices: an overview. *In-vitro Cellular & Developmental Biology-Plant*, **55** : 242 - 257.

Pooja, B. and Sheshshayee, M. S., 2017, Analysis of trait introgressed back cross progenies to identify superior lines for aerobic cultivation in rice (*Oryza Sativa* L.). *Mysore J. Agric. Sci.,* **51** (3) : 501 - 505.

Rukmini, M., Rao, G. J. N. and Rao, R. N., 2013, Effect of cold pretreatment and phytohormones on anther. *J. Exp. Biol.*, **1** (2) : 69 - 76.

Samantaray, Sanghamitra, Jauhar Ali, Katrina Lc Nicolas, Jawahar Lal Katara, Ram Lakhan Verma, Parameswaran, C., Devanna B. N., Awadhesh Kumar, Byomkesh Dash and Sudhansu Sekhar Bhuyan., 2021, 'Doubled haploids in rice improvement : approaches, applications and future prospects.' In *Rice improvement: physiological, molecular breeding and genetic perspectives*, pp. : 425 - 447. Cham: Springer International Publishing,

Sanasam, D., Udaya Kumar, M., Shivakumar, N. and Mohan Raju, B., 2019, Intra-line uniformity and inter-line variability of doubled haploid rice lines under different environmental conditions. *Plant Physiology Reports*, **24** : 15 - 23.

Sarao, N. K. and Gosal, S. S., 2018, *In-vitro* andro genesis for accelerated breeding in rice. *Biotechnologies of crop improvement,* **1** : *cellular approaches*, pp. : 407 - 435.

Sen, C. and Singh, R. P., 2011, Anther culture response in boro rice hybrids. *Asian J. Biotechnol*, **3** : 470 - 477.

Valera, H. and Pede, V., 2023, What do we know about the future of rice in relation to food system transformation. CGIAR Initiative on Foresight. *CGIAR News*, 2 February.

Wang, M., Duan, H., Jiang, G. and Li, Z., Research progress of rice anther culture technology. *Chin. Agric. Sci. Bull.* **38** : 18 - 22.

Zapata-Arias, F. J., 2003, Laboratory protocol for anther culture technique in rice. In *Doubled haploid production in crop plants: A manual* pp. : 109 - 116, Dordrecht: Springer Netherlands.