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Anther Culture Responses and Regeneration Efficiency in Five Indigenous Rice Genotypes of Bangladesh

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Key words: Anther culture, Albinism, Embryoids, Doubled haploids, Indigenous rice genotypes, Regeneration efficiency

Abstract

Anther culture is an efficient and reliable approach for the rapid production of doubled haploids (DHs) in rice and other cereal crops, enabling complete homozygous plants and accelerating genetic improvement. This study evaluated the androgenic responses of nineteen indigenous rice (*Oryza sativa* L.) genotypes from Bangladesh aiming to optimize the induction of embryoids, plant regeneration, and rooting efficiency. Among these materials Desi Lomba Atob, Chinigura, Krisnokoli, Buchi, and Surma exhibited positive callus responses. The highest number of embryoids (30.46 ± 0.75%) was achieved on N6 medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l Kn, while the greatest shoot regeneration (20.80 ± 1.28%) was obtained in Krisnokoli on MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA. Krisnokoli also yielded the highest frequency of green plant regeneration, whereas Surma exhibited 100% albinism. Rooting was successfully induced on half-strength MS medium supplemented with 0.5 mg/l IBA, resulting in improved rooting efficiency. These findings highlight the strong genotypic influences on androgenic efficiency and emphasize the importance of hormonal optimization for DHs production. The optimized protocol and the superior response of Krisnokoli provide a valuable foundation for the conservation and genetic improvement of indigenous rice genotypes in Bangladesh through anther culture.

Introduction

Rice (*Oryza sativa* L.) plays a pivotal role in global food production (Wei et al. 2024). It stands as the cornerstone of global food security, serving as the primary dietary staple for more than 50% of the world's population (Purwoko et al. 2025). Reports indicate that nearly 40% rice will be required to meet future food demand, emphasizing the urgent need for high-yielding, stress-tolerant varieties that can withstand drought, flooding and possess genetic stability (Khush 2005). The conventional breeding approach

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for producing homozygous lines through repeated selfing is inherently time-consuming, often requiring six to eight generations to achieve genetic fixation (Otani et al. 2005, Islam 2010c). In contrast, DH technology offers a faster and more efficient alternative, enabling the production of completely (100%) homozygous lines within a single generation, thereby accelerating varietal development and enhancing selection efficiency (Islam 2010a, Germanà 2011, Ingle et al. 2023). Several methods (e.g., anther and microspore culture) have been developed for the *in vitro* production of DHs and these have been used in breeding programs of many plant species (Redha et al. 2000, Nadim et al. 2024, An et al. 2025, Chen et al. 2025). DH breeding through anther culture has emerged as a powerful and practical alternative to conventional breeding for crop improvement (Purwoko et al. 2010, Islam and Tuteja 2012). Among various DH production techniques, androgenesis particularly via anther culture has proven to be one of the most effective biotechnological approaches for cereal crops, including rice (Raina and Zapata 1997, Islam 2010b, Khatun and Islam 2010, Redha and Islam 2010, Haque and Islam 2014, Ali et al. 2021).

The success of androgenesis depends on two critical stages: (i) the induction of embryogenic callus from microspores, and (ii) the regeneration of fertile green plants from anther or microspore derived calli. These stages are influenced by multiple factors such as genotype, microspore developmental stage, physiological condition of the donor plant, media composition, the balance of growth regulator, and culture environment (Jain et al. 1997, Islam et al. 2001, Tripathy et al. 2019, Haque and Islam 2024). Nevertheless, *indica* rice genotypes are often more recalcitrant to androgenesis compared with *japonica* types, exhibiting lower callus induction frequencies, poor regeneration potential, and a high incidence of albino plantlets chlorophyll-deficient regenerants that fail to survive (Raina and Zapata 1997, Islam et al. 2013, Gajecka et al. 2021). Overcoming these challenges requires strategic optimization of medium composition, growth regulator concentrations, and careful genotype selection to increase green plant regeneration efficiency and to minimize albino plants. Additionally, the physiological condition of the donor plant has a profound influence on androgenic response, with field-grown plants generally demonstrating better culture ability compared to those cultivated under greenhouse conditions (Kunz et al. 2000, Veeraraghavan 2007). The anther culture technique in rice breeding provides a rapid pathway to achieve plant homozygosity and homogeneity (Islam et al. 2023).

Bangladesh possesses an extraordinary diversity among indigenous aromatic rice genotypes, many of which are now less prevalent due to low yield potential, limited adaptability, and the displacement by modern varieties. These traditional landraces, such as Krisnokoli, Chinigura, Surma, Desi Lomba Atob and Buchi are highly prized for their distinctive aroma, grain quality, and cultural importance. Conservation and genetic enhancement of these varieties through advanced biotechnological methods, such as anther culture-based DH plant production, are essential for sustaining food security, preserving genetic diversity, and maintaining the cultural and economic heritage of

Bangladesh. Hence, the present study was undertaken to assess the *in vitro* androgenic responses of several indigenous aromatic rice genotypes of Bangladesh. The expected outcomes will contribute to the rapid development of DH lines, facilitating the production of pure and improved aromatic rice varieties with significant potential for sustainable rice improvement in Bangladesh.

Materials and Methods

This study was carried out using nineteen indigenous rice genotypes of Bangladesh, namely Chinigura, Radhuni Pagal, Desi Lomba Atob, Tulsimala, Buchi, Krisnokoli, Katarivog, Dolagura, Jirashail, Kolomkathi, Sonashail, Kajolota, Surma, Vasamanik, Dadkhani, Uknomodhu, Kotoktara, Jlonkhathi, and Ranashail. All experiments were conducted at the Plant Genetic Engineering and Biotechnology Laboratory and the research field of the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh.

Seeds of the nineteen different genotypes were grown in the IBSc research field under standard agronomic practices. Panicles were collected from the primary tillers at the booting stage, when the anthers were at the mid to late-uninucleate stage, an optimum stage for androgenesis. Spikes were harvested at early in the morning between 07:00 and 09:00 am when the distance between the base of the flag leaf and the primary leaf node was 5-10 cm (Ali et al. 2021). The freshly collected field-grown panicles were wrapped in moist sterile paper towels and stored at 4°C for 5-12 days to break dormancy and synchronize pollen development prior to inoculation.

After the cold pretreatment, panicles were surface sterilized under aseptic conditions in laminar airflow cabinet. This process included immersion in 70% (v/v) ethanol for 1 min, followed by treatment with 1-2% (v/v) sodium hypochlorite (NaOCl) solution for 10-15 min, and finally rinsed three to four times with sterile distilled water to remove residual sterilizing agents. Anthers were aseptically excised from the spikelets using sterile forceps and scissors. Each spikelet was held from the tip and cut at the base to detach the anther lobes from the filaments. Around sixty anthers were inoculated onto the induction medium in sterile Petri dishes, where each culture vessel represented one replication, and five replications were maintained for each treatment.

Embryoids induction was carried out using MS and N6 (Chu et al. 1975) media. These media were supplemented with various concentrations (T_0-T_{10}) of 2,4-dichloro phenoxyacetic acid (2,4-D) ranging from 1.0 to 3.0 mg/l, either single or in combination with NAA and Kn. Maltose was used as the carbohydrate source instead of sucrose, as it has been reported to improve androgenic response and reduce albinism in cereals (Tripathy et al. 2019). For plant regeneration, actively growing embryogenic calli were transferred to MS medium supplemented with different combinations of BAP, NAA, and Kn to determine the most effective hormonal composition. The combinations (R_0 - R_{10}) tested included BAP alone, BAP + NAA, BAP + Kn, and BAP + NAA + Kn. The pH of the

medium was adjusted to 5.8 before autoclaving at 121°C for 20 min under 15 psi pressure. For callus induction the cultures were incubated in complete darkness at 25 ± 2°C and 65% relative humidity for 4-8 weeks. The formation and proliferation of callus were observed periodically, and calli of approximately 2-3 mm in diameter were transferred to regeneration medium. For shoot regeneration from the callus the culture vessels were incubated at 25 ± 2°C under a 14 hrs photoperiod (2000-3000 lux) and 10 hrs of darkness. Regenerated shoots with a height of 5 cm or more were transferred to half-strength MS medium, either hormone-free or supplemented with low concentrations of auxins, to promote root induction. The medium was solidified with 0.4% gelrite, and rooting was achieved within 3-4 weeks under the same temperature and light conditions as during regeneration. The plantlets were acclimatized in a greenhouse under controlled temperature and humidity and later transferred to field conditions for further growth and seed set.

Data were collected at different stages of culture after 4-8 weeks of anther inoculation for callus induction, 3-4 weeks for shoot regeneration, and 3 weeks for root induction. The experiments were arranged in a completely randomized design (CRD) with five replications per treatment to minimize experimental error. Statistical analysis was performed using Microsoft Excel and SPSS software (Version 25). Mean values were compared using standard statistical tests, and differences among treatments were considered significant at the 5% probability level ($p < 0.05$).

Results and Discussion

The present investigation was aimed to evaluate the callus induction, regeneration, and rooting responses following the anther culture of nineteen indigenous rice genotypes. Among these, only five genotypes *viz*. Desi Lomba Atob, Chinigura, Krisnokoli, Buchi, and Surma demonstrated positive responses towards callus induction, highlighting the significant genotype dependence of androgenic potential (Fig. 1). Callus induction (CI) frequency was found to be varied significantly among these genotypes as well as between the MS and N6 basal media supplemented with different combinations of plant growth regulators (Table 1). Previous studies have shown that *indica* rice cultivars generally exhibit lower androgenic potential compared to *japonica* types (Raina 1997). Guha-Mukherjee (1973) observed that only 5 out of 18 *indica* cultivars showed pollen callusing, with just four producing plants, while Lentini et al. (1995) reported that only one out of 35 *indica* cultivars formed calli on N6 medium.

Among basal media, N6 and MS formulations are most commonly used for callus induction and plant regeneration, respectively (Lentini et al. 1995, Raina and Zapata 1997, Zaman and Islam 2024, Sarkar and Islam 2025). The proper combination of auxins such as 2,4-D + naphthalene acetic acid (NAA) and cytokinins such as 6-benzylamino purine (BAP) + Kn has been reported to enhance callus proliferation and promote efficient shoot regeneration (Chen et al. 1991, Mayakaduwa and Silva 2019).

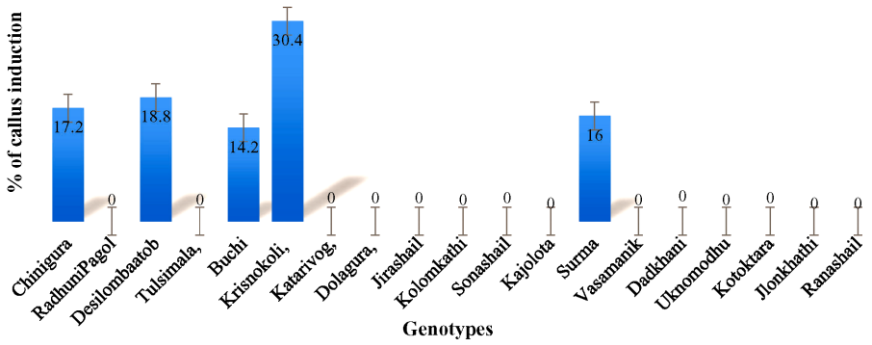

Fig. 1. Callus induction response of nineteen indigenous rice genotypes in N6 (T5) medium.

Table 1. Callus induction percentage of anther culture of five responsive indigenous rice varieties in MS and N6 media.

Treatments	Media	% of Callus Induction in different rice varieties (after 6th week of inoculation)				
		Desi Lomba Atob	Chinigura	Krisnokoli	Buchi	Surma
T_0 (Control)	MS	-	-	-	-	-
	N6	-	-	-	-	-
T_1	MS	1.80 ± 0.20^{k}	1.40 ± 0.24^{i}	5.00 ± 0.84^{l}	0.80 ± 0.37^{n}	1.20 ± 0.20^{k}
	N6	3.20 ± 0.20^{ijk}	2.60 ± 0.40^{hi}	8.00 ± 0.71^{k}	1.40 ± 0.24^{mn}	2.40 ± 0.24^{jk}
T_2	MS	5.20 ± 0.58^{ghi}	4.20 ± 0.58^{fgh}	9.00 ± 0.84^{jk}	2.80 ± 0.37^{klm}	3.20 ± 0.58^{ij}
	N6	6.60 ± 0.71^{fgh}	6.60 ± 0.51^{cd}	11.20 ± 0.80^{ij}	4.20 ± 0.37^{jk}	5.20 ± 0.20^{fg}
T_3	MS	7.20 ± 0.93^{efg}	5.00 ± 0.71^{defg}	13.40 ± 0.68^{ghi}	3.20 ± 0.37^{jkl}	4.60 ± 0.51^{fgh}
	N6	11.00 ± 0.49^{c}	9.60 ± 0.93^{b}	16.80 ± 0.37^{ef}	7.60 ± 0.51^{fg}	8.80 ± 0.37^{e}
T_4	MS	9.00 ± 0.89^{cde}	7.00 ± 0.71^{cd}	15.60 ± 0.81^{fg}	6.80 ± 0.86^{gh}	6.00 ± 0.45^{f}
	N6	14.20 ± 0.86^{b}	10.40 ± 1.08^{b}	22.80 ± 0.86^{b}	9.20 ± 0.86^{cde}	11.40 ± 0.75^{bc}
T_5	MS	11.20 ± 0.97^{c}	10.60 ± 0.68^{b}	20.00 ± 0.71^{cd}	9.80 ± 0.58^{cd}	9.00 ± 0.55^{de}
	N6	18.80 ± 1.07^{a}	17.20 ± 0.86^{a}	30.46 ± 0.75^{a}	14.20 ± 0.58^{a}	16.00 ± 0.71^{a}
T_6	MS	9.60 ± 0.87^{cd}	7.00 ± 0.71^{cd}	16.20 ± 0.58^{f}	8.60 ± 0.51^{def}	7.80 ± 0.58^{e}
	N6	16.20 ± 0.97^{b}	11.60 ± 0.75^{b}	21.80 ± 0.58^{bc}	12.20 ± 0.66^{b}	12.60 ± 0.68^{b}
T_7	MS	8.00 ± 0.71^{def}	6.40 ± 0.51^{cd}	14.40 ± 1.03^{fgh}	7.80 ± 0.37^{efg}	5.40 ± 0.51^{fg}
	N6	11.20 ± 0.80^{c}	10.40 ± 0.40^{b}	18.80 ± 0.86^{de}	10.40 ± 0.40^{c}	11.40 ± 0.93^{bc}
T_8	MS	6.60 ± 0.51^{fgh}	4.60 ± 0.68^{efgh}	11.20 ± 0.80^{ij}	5.80 ± 0.37^{hi}	4.20 ± 0.58^{gh}
	N6	9.60 ± 0.68^{cd}	7.80 ± 0.58^{c}	16.80 ± 1.16^{ef}	8.40 ± 0.24^{def}	10.40 ± 0.51^{cd}
T_9	MS	5.20 ± 0.80^{fhi}	4.20 ± 0.37^{fgh}	10.00 ± 0.71^{jk}	4.20 ± 0.58^{jk}	3.40 ± 0.51^{ij}
	N6	7.40 ± 0.75^{defg}	5.80 ± 0.49^{cd}	12.60 ± 0.51^{hi}	6.80 ± 0.37^{gh}	8.60 ± 0.51^{e}
T_{10}	MS	2.60 ± 0.40^{jk}	1.80 ± 0.37^{i}	9.00 ± 0.84^{jk}	1.80 ± 0.37^{lmn}	2.40 ± 0.24^{jk}
	N6	4.40 ± 0.75^{hij}	3.20 ± 0.58^{ghi}	10.00 ± 0.71^{jk}	4.40 ± 0.51^{ij}	6.20 ± 0.37^{f}

T_0 (Control) = (Without growth regulators), T_1 = (2,4-D 1.0 mg/l), T_2 = (2,4-D 2.0 mg/l), T_3 = (2,4-D 3.0 mg/l), T_4 = (2,4-D 1.0 mg/l + Kn 0.5 mg/l), T_5 = (2,4-D 2.0 mg/l + Kn 0.5 mg/l), T_6 = (2, 4-D 2.0 mg/l + Kn 1.0 mg/l), T_7 = (2,4-D 1.0 mg/l + NAA 1.0 mg/l + Kn 0.5 mg/l), T_8 = (2,4-D 1.0 mg/l + NAA 1.0 mg/l + Kn 1.0 mg/l), T_9 = (2,4-D 2.0 mg/l + NAA 1.0 mg/l + Kn 0.5 mg/l), T_{10} = (2,4-D 2.0 mg/l + NAA 1.0 mg/l + Kn 1.0 mg/l).

Mean values followed by same lower-case letters (a, b etc.) are not significantly different ($p < 0.05$) among different treatments within the same genotypes on MS and N6 media, as determined by Duncan's Multiple Range Test (DMRT).

No callus formation occurred in the control treatment (T_0) on either medium, confirming the necessity of exogenous hormone supplementation for callogenesis. The addition of auxins and cytokinins markedly enhanced callus formation, with the response generally increasing up to an optimal concentration and then declining slightly at higher doses (Fig. 1). Among all treatments, T_5 (N6 medium) produced the highest callus induction (30.46 ± 0.75%) in Krisnokoli, followed by Desi Lomba Atob (18.80 ± 1.07%), Chinigura (17.20 ± 0.86%), Surma (16.00 ± 0.71%), and Buchi (14.20 ± 0.58%). The lowest callus induction was recorded in Buchi (0.80 ± 0.37%) and Surma (1.20 ± 0.20%) on MS medium in T_1 (Table 1). The cultures were examined weekly up to 8 weeks and the callus induction frequency was recorded on the 6th week (Fig. 2).

Fig. 2(a-j). Process of anther culture derived plantlet production in Krisnokoli rice genotype: (a) inoculation of anthers onto induction medium, (b) callus initiation from anthers (arrow indicates initiation of embryoids), (c-e) regeneration of plantlets from the callus (arrow shows shoot initiation and formation of albino plantlets), (f-g) rooting of plantlets, (h-i) plant acclimatization and establishment in pot mixture, and (j) field grown plants.

Overall, N6 medium outperformed than MS, indicating that it's optimized nitrogen composition and organic nutrients were more conducive to androgenic callus formation. The genotype Krisnokoli produced compact, friable, creamy-white calli, while Buchi and Surma showed smaller, less friable calli, reflecting their lower morphogenic capacity. These findings agree with earlier observations that androgenic response in rice is largely genotype-specific (Khatun et al. 2010). Consistent with the present findings, N6 medium

induced the highest callusing in Taraori Basmati (Grewal et al. 2006) and in F_1 populations of inter-varietal crosses (Min et al. 2016). Additionally, N6 and MS were found to be complementary, N6 promoting callus formation, while MS favored green-shoot regeneration (Khatun et al. 2012, Rout et al. 2016, Naik et al. 2017, Lantos et al. 2022).

Following successful callus induction, the embryogenic calli (2-3 mm) were sub-cultured onto regeneration media containing varying concentrations of BAP, NAA, and Kn on MS medium. The regeneration frequency and plantlet quality varied markedly among genotypes and treatments (Table 2, Fig. 1). The most effective regeneration was achieved on MS medium supplemented with BAP (2.0 mg/l) + NAA (0.5 mg/l) + Kn (0.5 mg/l), specifically in the R_6 treatment,which resulted in the highest shoot regeneration (20.80 ± 1.28) in Krisnokoli. This was followed by Desi Lomba Atob and Chinigura, while Surma showed the lowest regeneration response (12.20 ± 1.20). The combination of BAP and NAA proved synergistic, promoting both shoot elongation and green plant differentiation, while higher cytokinin levels occasionally induced vitrification and reduced shoot quality. The genotype Krisnokoli demonstrated the highest proportion of green plant regeneration (Fig. 3), indicating its superior embryogenic competence and high responsiveness to auxin-cytokinin balance. These findings align with earlier observations, reported by Dash et al. (2022), who found similar result in rice anther culture. Conversely, Surma produced 100% albino plantlets, which exhibited white or pale-yellow leaves due to impaired chloroplast biogenesis, a phenomenon commonly associated with albinism in *indica* rice androgenesis (Niizeki and Oono 1968, Khatun et al. 2018).

Table 2. Percentage of plant regeneration from anther-derived calli of five indigenous rice genotypes.

Treatments	% of Plant regeneration in different rice varieties (after 4 weeks)				
	Desi Lomba Atob	Chinigura	Krisnokoli	Buchi	Surma
R_0 (Control)	-	-	-	-	-
R_1	0.80 ± 0.20^{g}	0.80 ± 0.20^{f}	1.00 ± 0.00^{f}	0.20 ± 0.20^{f}	0.00 ± 0.00^{e}
R_2	2.20 ± 0.37^{f}	1.60 ± 0.40^{f}	3.60 ± 0.24^{e}	0.80 ± 0.20^{f}	0.20 ± 0.20^{e}
R_3	7.60 ± 0.40^{d}	5.00 ± 0.32^{de}	9.20 ± 0.58^{d}	3.60 ± 0.40^{e}	2.60 ± 0.40^{d}
R_4	9.80 ± 0.37^{c}	8.40 ± 0.51^{c}	12.40 ± 0.60^{c}	5.80 ± 0.20^{d}	4.60 ± 0.60^{c}
R_5	14.60 ± 0.81^{b}	12.60 ± 0.68^{b}	15.20 ± 1.24^{b}	9.00 ± 0.71^{c}	8.60 ± 0.68^{b}
R_6	**17.00 ± 0.55^{a}**	**15.40 ± 0.51^{a}**	**20.80 ± 1.28^{a}**	**13.60 ± 0.68^{a}**	**12.20 ± 1.20^{a}**
R_7	15.20 ± 0.37^{b}	13.00 ± 0.32^{b}	14.20 ± 0.80^{bc}	10.60 ± 0.51^{b}	9.20 ± 0.73^{b}
R_8	9.60 ± 0.40^{c}	7.80 ± 0.37^{c}	9.60 ± 0.40^{d}	5.00 ± 0.32^{d}	4.60 ± 0.40^{c}
R_9	8.20 ± 0.37^{d}	6.20 ± 0.37^{d}	7.60 ± 0.51^{d}	3.20 ± 0.37^{e}	3.00 ± 0.45^{cd}
R_{10}	4.60 ± 0.51^{e}	4.00 ± 0.45^{e}	5.20 ± 0.37^{e}	2.60 ± 0.60^{e}	2.40 ± 0.51^{d}

R_0 (Control) = Without growth regulators, R_1 = BAP 1.0 mg/l, R_2 = BAP 2.0 mg/l, R_3 = BAP 3.0 mg/l, R_4 = BAP 1.0 mg/l + NAA 0.5 mg/l, R_5 = BAP 2.0 mg/l + NAA 0.5 mg/l, R_6 = BAP 2.0 mg/l + Kn 0.5 mg/l + NAA 0.5 mg/l, R_7 = BAP 2.0 mg/l + Kn 1.0 mg/l + NAA 0.5 mg/l, R_8 = BAP 2.0 mg/l + Kn 1.0 mg/l + NAA 1.0 mg/l, R_9 = BAP 1.0 mg/l + Kn 2.0 mg/l + NAA1.0 mg/l, R_{10} = BAP 0.5 mg/l + Kn 2.0 mg/l + NAA 1.0 mg/l.

Mean values followed by different lowercase letters (a, b, etc.) within the same genotype are significantly different at $p < 0.05$ across the treatments.

The observed variation in green and albino plant frequency among genotypes suggests that chloroplast development and plastid differentiation are strongly influenced by genetic control. Similar findings were reported by Raina (1997) and Chen et al. (2011), who emphasized that nuclear-cytoplasmic interactions and gene expression stability influence the recovery of photosynthetically active green regenerants. The superior green regeneration observed in Krisnokoli therefore reflects its robust genetic makeup and favorable cytoplasmic compatibility for haploid plant development.

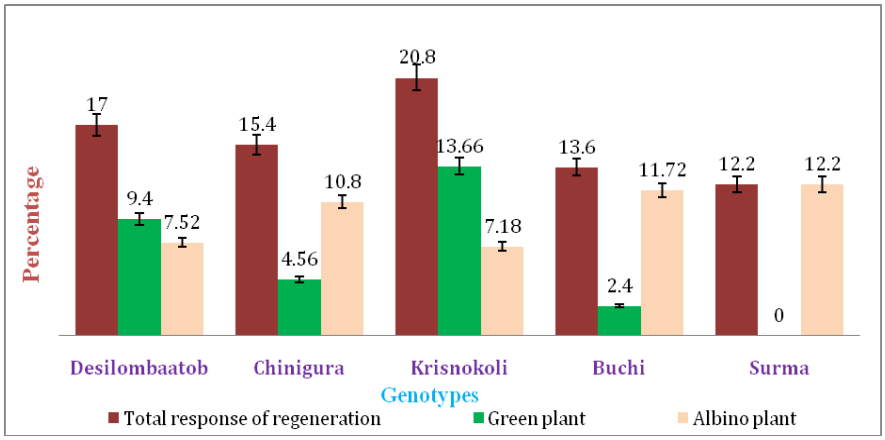

Fig. 3. Percentage of plant regeneration in different rice genotypes, highlighting the proportion of green and albino plants.

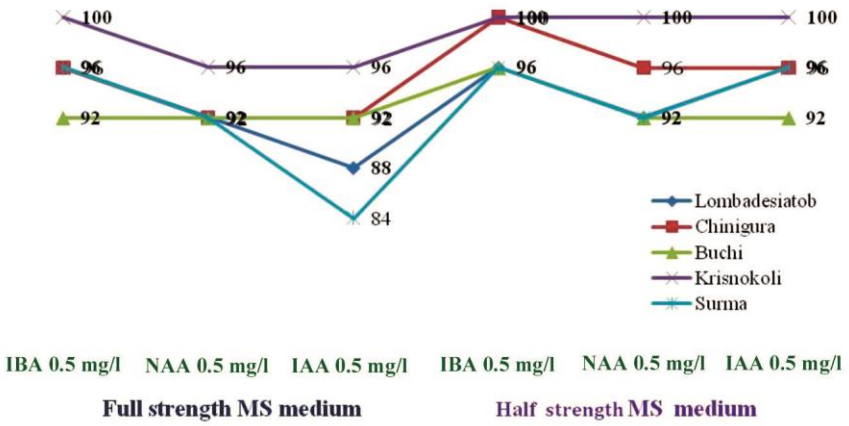

Fig. 4. Percentage of rooting in different rice genotypes on MS and ½MS media supplemented with various concentrations of auxins.

Additionally the rooting performance of regenerated plantlets also varied among genotypes and auxin treatments (Fig. 4). Among the three auxins tested, IBA, NAA, and IAA, IBA proved most effective, producing the highest rooting percentage and root

number per plantlet. The genotype Krisnokoli achieved 100% rooting in IBA, producing an average of 66.67 ± 0.71 roots per plantlet, followed by Desi Lomba Atob (60.67 ± 0.42) and Buchi (54.17 ± 0.83). Comparatively lower responses were obtained using NAA and IAA, where the number of roots ranged from 33.33 to 62.50 per plantlet (Table 3, Fig. 2f-g). Fully developed plantlets were acclimatized and were established under field condition (Fig. 2i-j).

Table 3. Effects of different auxins on rooting in anther derived plantlets in five indigenous rice genotypes.

Genotypes	IBA (0.5 mg/l)		NAA (0.5 mg/l)		IAA (0.5 mg/l)	
	Number of root per micro-shoots ($\bar{X}$ ± S.E.)	Average length of root (cm) ($\bar{X}$ ± S.E.)	Number of root per micro-shoots ($\bar{X}$ ± S.E.)	Average length of root (cm) ($\bar{X}$ ± S.E.)	Number of root per micro-shoots ($\bar{X}$ ± S.E.)	Average length of root (cm) ($\bar{X}$ ± S.E.)
Desi Lomba Atob	54.17 ± 0.83^{c}	3.13 ± 0.19^{c}	49.50 ± 1.28^{c}	3.03 ± 0.09^{b}	51.33 ± 0.56^{c}	3.10 ± 0.06^{b}
Chinigura	60.67 ± 0.42^{b}	3.10 ± 0.15^{c}	55.67 ± 0.49^{b}	2.92 ± 0.12^{bc}	58.28 ± 1.20^{b}	2.93 ± 0.03^{bc}
Krisnokoli	**66.67 ± 0.71^{a}**	**3.44 ± 0.29^{b}**	**61.33 ± 1.73^{a}**	**3.17 ± 0.33^{a}**	**62.50 ± 0.43^{a}**	**3.47 ± 0.33^{a}**
Buchi	49.67 ± 0.95^{d}	3.33 ± 0.33^{b}	42.33 ± 0.76^{d}	2.67 ± 0.18^{d}	46.50 ± 0.92^{d}	2.77 ± 0.12^{c}
Surma	38.33 ± 0.84^{e}	3.83 ± 0.33^{a}	33.33 ± 0.76^{e}	2.87 ± 0.09^{c}	35.33 ± 0.67^{e}	3.43 ± 0.13^{a}

Statistical significance among treatments was determined using one-way ANOVA followed by DMRT at $p < 0.05$. Different letters (a,b,c,d) indicate statistically significant differences among means.

These results of this study are consistent with the findings of Al Noor et al. (2019), who reported that IBA promotes a more extensive root system due to its higher stability and efficient auxin transport compared to NAA and IAA. Well-developed roots are essential for acclimatization and subsequent transfer to soil, ensuring the survival and fertility of DHs plants. The combined findings from callus induction, regeneration, and rooting experiments revealed substantial genotypic variability among the studied indigenous rice genotypes. Among them, Krisnokoli consistently exhibited superior performance in callus formation, green plant regeneration, and rooting efficiency, suggesting its potential as a promising model genotype for DHs development through anther culture. In contrast, Buchi and Surma showed lower androgenic efficiency and higher albinism frequencies, indicating possible genetic or cytoplasmic limitations. These findings corroborate the observations of Purwoko et al. (2010) emphasizing that anther culture based doubled haploid breeding provides a rapid and reliable pathway to achieve complete homozygosity and accelerate pure line development in rice. Therefore, optimizing genotype selection, hormonal composition, and culture media formulation can substantially enhance the efficiency of DH production in *indica* rice breeding programs. Overall, the outcomes of this study contribute valuable insights toward the quick development of pure lines from indigenous rice genotypes through improved anther culture techniques.

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