A Seminar Paper on

Prospects and Challenges of Colchicine-based Polyploidy Induction in Rice

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Submitted to

Course Instructors

1. Dr. A. K. M. Aminul Islam

Professor,

Department of Genetics and Plant Breeding, BSMRAU

2. Dr. Satya Ranjan Saha

Professor,

Department of Agroforestry and Environment, BSMRAU

3. Dr. Shaikh Shamim Hasan,

Professor,

Department of Agricultural Extension and Rural Development, BSMRAU

4. Dr. Dinesh Chandra Shaha,

Associate Professor,

Department of Fisheries Management, BSMRAU

Major Professor

Dr. Mohammad Sharif Raihan

Professor,

Department of Genetics and Plant Breeding, BSMRAU

Submitted by

Akbar Hossain

Reg. no: 17-05-4202

MS student, Department of Genetics and Plant Breeding, BSMRAU

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY, GAZIPUR-1706

ABSTRACT

Prospects and Challenges of Colchicine-based Polyploidy Induction in Rice[1]

By

Akbar Hossain[2]

The technique of colchicine-based polyploidy induction is a well-established method for crop improvement and diversification. But different crops have different application techniques. This review described the current challenges and prospects of colchicine-based polyploidy induction in rice. The different methods used for colchicine application and their impacts on induction efficiency and genetic stability also discussed. There are three methods among them, the most efficient treatment raised by the callus method ranges from 31% to 65.5%. Additionally, potential benefits of polyploidization in rice, such as enhanced stress tolerance at water potential -40 kPa and improved grain quality, new genetic diversity, more photosynthesis capacity increase nutritional quality such as seed protein content in four diploid lines ranged from 8.2% to 9.3%, while their tetraploids had higher protein content ranging from 9.6% to 11.2% also discussed. Although polyploid rice has some disadvantages, such as during meiosis 125 genes are down-regulated, inhibiting normal pollen development, abnormal meiosis does happen, and genetic stability is also lower in polyploid rice. Recent advancements in genome editing with CRISPR/Cas9 technology for deletion of harmful gene in rice such as manipulation of the nt-TMS9-1 gene helps restore pollen fertility. In conclusion, while colchicine-based polyploidy induction in rice faces several challenges, it presents a promising avenue for crop improvement, and advancements in plant breeding and genomic resources offer exciting prospects for further improvements.

Key words: Polyploid, Colchicine, Autotetraploid rice, Genetic stability.

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[1] A seminar paper presented for seminar course GPB 598; winter'2022.

[2] MS Student, Department of Genetics and Plant Breeding, BSMRAU, Gazipur 1706.

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CHAPTER 1

INTRODUCTION

Rice, also known as *Oryza sativa*, is a member of the grass family Poaceae (Matthew *et al*., 2007). Rice is divided into three subspecies: indica, japonica, and javanica, each with unique characteristics and uses (Yang *et al*., 2014). Rice is a staple food for over 90% of people in Asia, providing iron, folic acid, thiamine, and niacin, but lacking in fibre and fat. It is also a good source of magnesium, phosphorus, manganese, and selenium (Fukagawa & Ziska, 2019). However, with the human population rapidly increasing, feeding the projected 9.1 billion people by 2050 presents a major challenge that puts immense pressure on all natural resources (How to Feed the World, 2050). Rice production is threatened by decreasing cultivable land, a shortage of irrigation water, increasing temperature and carbon dioxide, susceptibility to disease and insect pests, and other factors. Currently, diploid rice is the most widely cultivated, but compared with polyploid crops like wheat, rice has a smaller genome and lower DNA content. The genetic resources of cultivated diploid rice are limited, hindering further development of rice breeding (Jiang *et al*., 2018).

Polyploidy, which refers to having more than two complete sets of chromosomes in a cell nucleus, is a common occurrence in plant evolution and diversification (Soltis *et al*., 1999). Auto and allopolyploidy can both be found in nature (Yang *et al*., 2019), with most angiosperms, including many crop plants, experiencing polyploidy between 50 and 70 percent of the time during their evolutionary process (Chen *et al*., 2007). Polyploid rice could help address the challenges that diploid rice faces today. Artificial polyploidy can be achieved through interspecific hybridization, in vitro endosperm culture, or colchicine-induced somatic cell doubling (Fatima *et al*., 2014). Colchicine is a toxic natural alkaloid extracted from the plant (*Colchicum autumnale*). It has been used for many years for medicinal purpose. However, it also has an interesting application in the field of plant breeding (Münzbergová, 2017). Colchicine is a potent mitotic inhibitor, meaning it stops cell division during mitosis, the process by which cells divide and replicate. This property makes it useful in inducing polyploidy in plants, which is the process of increasing the number of sets of chromosomes in a cell (Taylor, 1965).

Polyploidy has been successfully done artificially in many crop species, but for rice it is not well developed due to some barriers (Koide *et al*., 2020).

To induce polyploidy colchicine-base induction being mainly used in rice (Chen *et al*., 2021). Although polyploidy can interfere with normal meiosis and mitosis in rice, recent research on autotetraploid rice has primarily focused on photosynthetic characteristics, quality traits, DNA methylation, agronomic traits, seed setting rate, and yield potential (Chen *et al*., 2021). Further research is needed to overcome the drawbacks of poor seed germination rates and to exploit the benefits of polyploidy in grain quality, resistance, and biological yield.

Classical polyploidy methods like colchicine can be used to produce fertile tetraploid rice, which may be a promising concept for rice breeding (Koide *et al*., 2020). However, polyploidy can alter gene expression, potentially leading to harmful expression of genes (Wu *et al*., 2015). Thus, further research is necessary to fully understand the effects of polyploidy on rice and develop strategies to minimize negative effects.

Based on above facts the objectives of this reviewed paper are

- To assess different methods of polyploidy induction and confirmation in rice
- To evaluate the importance of polyploidy in rice
- To identify and address barriers associated with polyploidy in rice and their future prospects to overcome such problems

CHAPTER 2

MATERIALS AND METHODS

A scientific approach involves a thorough knowledge of the subject. This seminar paper is a review in its entirety because all information was collected from secondary sources. To write this seminar paper, all the information was taken from related scientific papers that had been published in various journals, reports, internet browsing. Valuable suggestions and information were received from honourable major professors and course instructors. To improve this work, all the information was gathered and then organized properly and chronologically.

CHAPTER 3

REVIEW OF FINDING

3.1 Polyploidy and its classification

Based on chromosome number, polyploidy can be classified into two categories: euploids and aneuploids.

3.1.1 Euploids

Organism with chromosomal numbers that are exactly multiples of the species' normal haploid number. Euploids can be further divided into either autopolyploids or allopolyploids depending on how the genome is comprised. The most prevalent class of euploids is tetraploid (Comai., 2005).

3.1.2 Autopolyploid

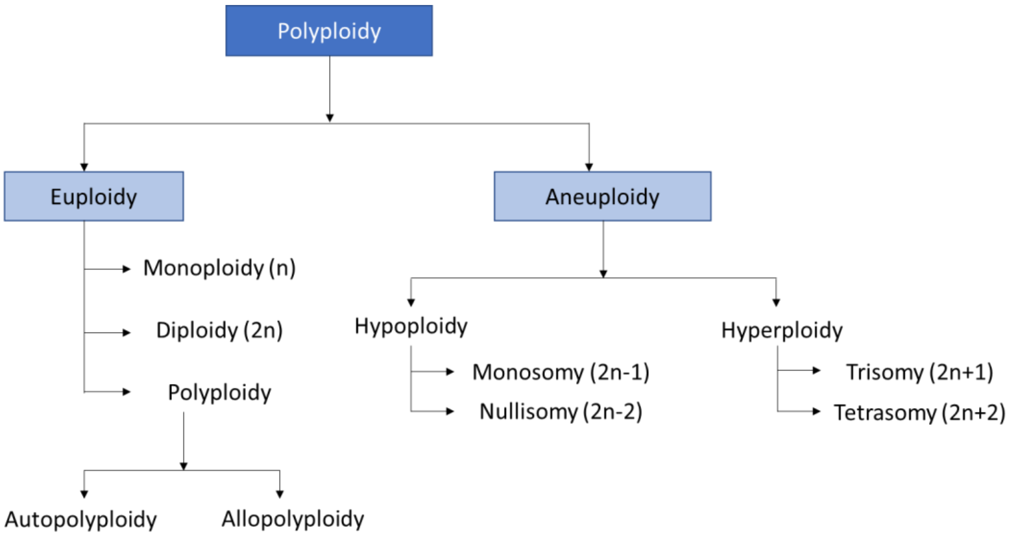
Autoploids are another name for autopolyploids. They have multiple copies of the sam e genome's basic set of chromosomes (x) (Chen, 2010). Autopolyploidy occurs naturally through the process of chromosome doubling. Forage and ornamental grasses have gained more vigour as a result of this chromosomal doubling process that occurs naturally (Acquaah, 2007). Breeders use the process of chromosomal doubling in vitro through induced polyploidy to generate superior crops because autopolyploidy has some advantages in nature. For example, rice has been made autopolyploid by using colchicine treatment, and certain advantages have been observed in rice after chromosome doubling (Koide *et al*., 2020).

3.1.3 Allopolyploid

A polyploid organism with multiple sets of chromosomes that come from different species known as Allopolyploid (Acquaah, 2007). Around half of the species in the genus *Oryza,* which includes rice, are allopolyploid. These species offer a special chance to research the evolution of polyploid species in addition to being essential resources for rice breeding (Zou *et al*., 2015). To transfer genes from another species, distant hybridization in rice through allopolyploidy can be a new door for rice breeding (Wang *et al*.,2005).

3.2 Different process and mechanism of polyploidy induction in rice

Physical induction, biological induction, and chemical induction are the three types of artificial polyploidy induction (Madani *et al.*, 2021). Polyploids can form during the physical induction procedure because of temperature shocks, irradiation, grafting, etc. But physical induction has low efficiency; hence, it is not commonly used. The three most commonly utilized chemicals are naphthalene pentane, naphthalene ethane, and naphthalene ethane. Among them, the most often used mutagen is colchicine in case of rice due to their high efficiency of polyploidy induction (Chen *et al*., 2021). When a rare mitotic or meiotic occurrence, such as nondisjunction, results in the generation of gametes or cells with a full complement of duplicate chromosomes, this condition is known as polyploidy. Colchicine is a chemical that interferes with the regular process of cell division and can cause polyploidy in plants. Colchicine prevents the development of the spindle fibres, which are necessary for chromosomal separation during cell division (Taylor, 1965). Colchicine helps induce polyploidy in this way, and the effects of colchicine can produce diploids, triploids, and tetraploids (Chen *et al*., 1945).



Source: (Chromosomal Mutations-Botany: Chromosomal Basis of Inheritance, 2023)

Fig.1. Numerical changes in chromosomes.

A triploid zygote is created when a diploid gamete combines with a haploid gamete, albeit these triploids are typically unstable and frequently sterile (Woodhouse *et al.*, 2009). Figure1 showed different ploidy level and colchicine can produce 1-10% triploids, pentaploids, and aneuploids in rice (Alemanno & Guiderdoni, 1994).

3.3 Methods of Polyploidy induction in Rice using colchicine

In rice, colchicine can be applied in different ways, and each experiment has different results. All experimental success depends on different dosages and methods. Here, various established methods and their dosages are reviewed.

3.3.1 Split application of colchicine to germinated rice seedlings

Colchicine was used to the germinated seedlings' coleoptile so that no chimera formed (Huang *et al*., 1995). As the coleoptile of rice was tightly linked, germinated seedlings were split with a sharp razor blade when they were 3–5 mm long, and they split into two halves up to the base, a cotton ball was placed. Following that, 0.01% and 0.05% colchicine were applied, and the treated seedlings were kept in a dark, damp chamber. The cotton was treated with colchicine the next day as well and removed 24 hours later. The optimal colchicine concentration for inducing polyploidy in rice appears to be between 0.05% and 0.1%.

Table1: Effect of Colchicine treatment (0.1 % colchicine solution)

Varieties	No. of seedling treated	No. of seedling survived after treatment	No. of individuals with 4x (auto tetraploid)
Aikoku	10	2	2
Konanto	10	6	4
Philippines No. 3	10	5	4
Russia no. 59	10	3	3

Source: (Luong, 1951)

Table2: Effect of Colchicine treatment (0.05 % colchicine solution)

Varieties	No. of seedling treated	No. of seedling survived after treatment	No. of individuals with 4x (auto tetraploid)
Rikuha no. I32	10	8	4
Ginboz	10	7	2
Asahi no. 1	10	9	1
Kairyo hagutai	10	9	2
Tanko hoir	10	7	1

Source: (Luong, 1951)

There were 90 treated seedlings in total, with 56 surviving. So the survival rate after treatment was 62%. But they found only 23 with a doubling of chromosomes, which was around 25% success. Between the treatments, 0.05% showed a higher survival rate than 0.1%, but 0.1% has a higher autotetraploid rate than that of 0.05%.

3.3.2 Methods of soaking rice seeds in colchicine

According to (Gaafar *et al*., 2017) the seeds were initially immersed in a corrosive sublimate solution. After that, the seeds were submerged in solutions containing 0.2% colchicine for 24 hours and 50 hours, respectively. The seeds were carefully cleansed with water before being placed in a Petri dish. Seeds were germinated in Petri dish and seedlings were selected from each treatment and planted separately in an earthen pot.

Table3: Number of survived seedlings after soaking in 0.2% colchicine

Colchicine treatment (h)	Total number of seeds	Number of survived seedlings	Survived seedlings (%)
24 hours	40	17	42.50
50 hours	40	6	15.5

Source: (Gaafar *et al*., 2017)

After that all survived seeds were taken to the main field and following traits were observed

Table4: Overall effects of colchicine after 50 hours of seed treatment

Line/plant	Yield/plant (g)	Seed set (%)	1000-grains weight (g)	Effective tillers/plant	Panicle length (cm)
P35	82.90	62.29	23.80	38.00	20.30
P36	20.20	68.11	16.90	28.00	20.00
P37	75.00	81.90	18.10	31.00	20.60
P38	27.00	81.90	24.00	22.00	20.80
P39	14.41	9.18	19.20	20.00	18.60
P40	86.60	75.72	20.90	21.00	20.00
Grand mean	51.02	63.18	20.48	26.67	20.05

Source: (Gaafar *et al*., 2017)

Table5: Overall effects of colchicine after 24 hours of seed treatment

Line/plant	Yield/plant (g)	Seed set (%)	1000-grains weight (g)	Effective tillers/plant	Panicle length (cm)
Egyptian Hybrid1	55.00	96.90	23.90	23.00	22.80
P18	50.95	13.41	20.28	29.00	20.00
P19	16.80	75.08	20.45	17.00	20.50
P20	39.50	16.82	23.25	35.00	20.00
P21	34.23	17.05	20.00	26.00	22.00
P22	33.00	13.80	27.90	35.00	20.10
P23	33.50	76.98	20.53	23.00	21.30
P24	151.80	86.44	22.90	35.00	22.00
P25	180.00	81.07	19.80	37.00	21.80
P26	74.70	90.19	20.57	28.00	22.20
P27	74.70	90.19	20.00	20.00	22.00
P28	84.67	69.68	21.62	28.00	21.00
P29	84.20	77.39	19.70	19.00	22.50
P30	78.10	61.30	21.58	32.00	22.20
P31	72.90	74.28	22.80	35.00	22.20
P32	23.20	7.48	21.20	38.00	22.00
P33	58.20	71.15	19.66	33.00	20.00
P34	61.50	71.95	22.30	32.00	19.30
Grand mean	67.76	58.49	21.04	24.47	21.24

Source: (Gaafar *et al.*, 2017)

Table 2,3 shows the overall influence of different soaking periods with 0.2% strength of colchicine solution on the rate of number of Yield/plant, Seed set, 1000- grains weight, Effective tillers/plant, Panicle length. The best performance was obtained after 24 hours of soaking, then 50 hours of soaking. Colchicine is effective in treating chromosomal abnormalities when used in the soaking method.

3.3.3 Application of colchicine to rice callus

According to (Perera & Dahanayake, 2016) colchicine was applied to two rice varieties, Sulaai and Suwadel, in vitro. After rinsing with surface sterilant, rice seeds were placed in a MS basal medium to initiate callus formation. The calluses were then exposed to various Colchicine concentrations (30 mg/L, 60 mg/L, 90 mg/L, and 120 mg/L) and kept for various times (0 h, 24 h, 48 h, and 72 h). Optimal light intensity and humidity were maintained. After root formation in the medium, the root tip was removed and examined under a microscope. They found some plants with polyploidy and some plants with no polyploidy. The control treatment and all colchicine treatments of 12 hours exposed callus and 24 hours exposed callus at 30, 60, and 90 mgL-1 were yellow in both rice genotypes. However, the 120 mgL-1 dose in 24 hours and the complete 78-hour treatment with colchicine turned brown.

Table 6: Number of days to regenerate plantlets and quantity of buds from callus

Hour	Colchicine conc. (mgL^{-1})	Average No. of buds		No. of days for shoot initiation
		Suwadel (after month)	Sulaai (after month)	
12	Control	6	8	22
	30	5	6	31
	60	4	5	35
	90	1	2	36
	120	0	0	-
24	30	3	4	32
	60	3	3	40
	90	1	1	42
	120	0	0	-

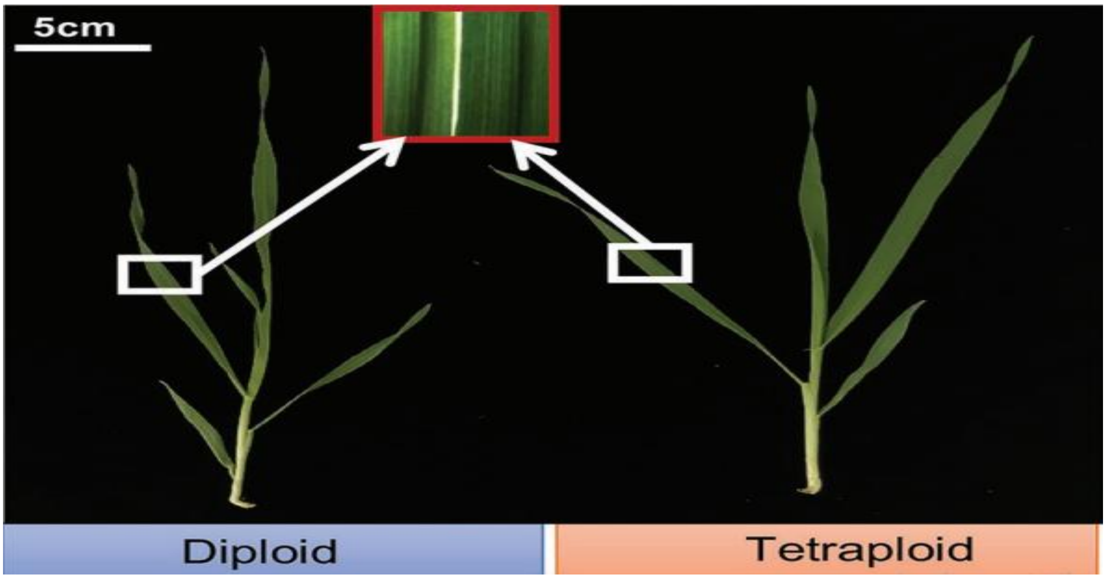
Source: (Perera & Dahanayake, 2016)

Duncan's multiple range test (P=0.05) determined that the same letter is not statistically different. The regeneration rates in the colchicine treatments were lower than in the control, particularly at greater concentrations and for longer periods of time. Media that contained colchicine took longer to regenerate the shoot bud compared to the controlled condition. Also, those media containing a high concentration of colchicine were delayed in producing the shoot bud compared to those containing a low concentration of colchicine (Dhahanayake, 2008).

3.4 Confirmation of autotetraploidy in rice

The number of chromosomes in the cells will increase due to the application of colchicine (Manzoor *et al*., 2019). Colchicine has induced polyploidy by counting the number of chromosomes in the cells of the treated plants compared to the control plants. The chromosome number of rice is 24 (Kurata *et al*., 2002), so if it has more than 24 chromosomes, it could be an indication of colchicine-induced polyploid rice. Flow cytometry is a technique for measuring the amount of DNA in cells. The treatment with colchicine increases the amount of DNA in the cells, which may be evaluated by flow cytometry (Moghbel *et al*., 2015). If the treated rice plants have more DNA than the control plants, this indicates that colchicine has caused polyploidy. Plant morphology could change as a result of colchicine-induced polyploidy.

The size and shape of the fruits, flowers, and leaves, for instance, may vary from those of the control plants.

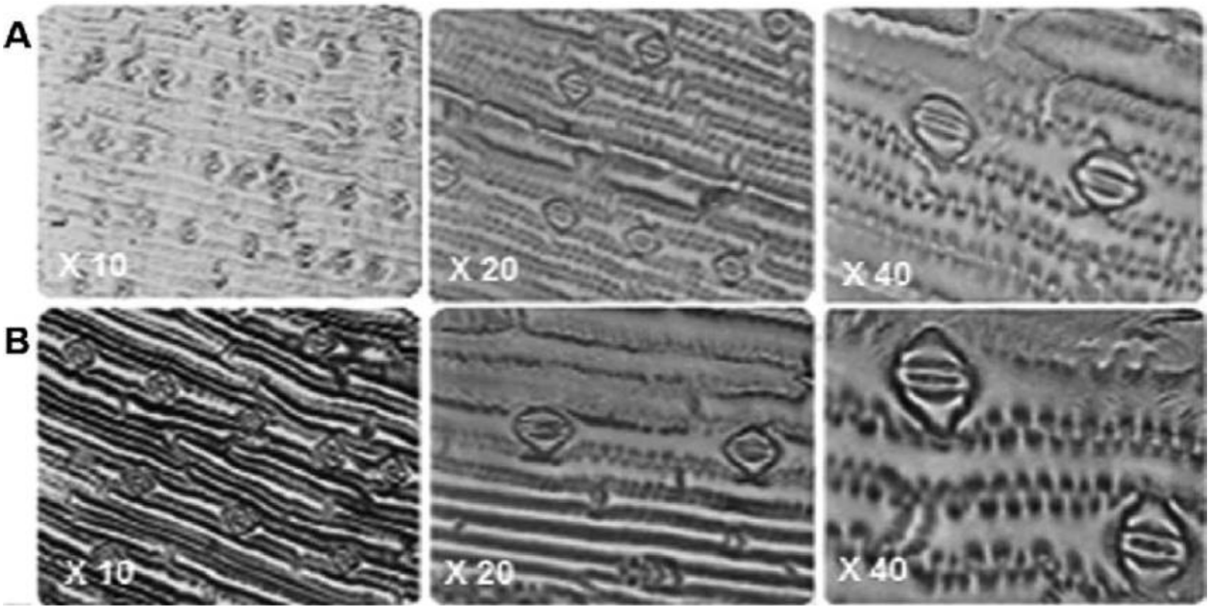


Source: (Yang *et al*., 2014)

Fig.2. Shoots of the diploid and tetraploid rice seedling.

From Figure 2, it can be seen that the colour and size of the tetraploid rice plant increased. As the colour increased, it indicated that there was more accumulation of leaf tissue and cellular content in the rice leaf.

When compared to untreated plants, plants that have had colchicine treatment may have more stomata per unit area of leaf surface (Moghbel *et al*., 2015).



Source: (Ardabili *et al*., 2015)

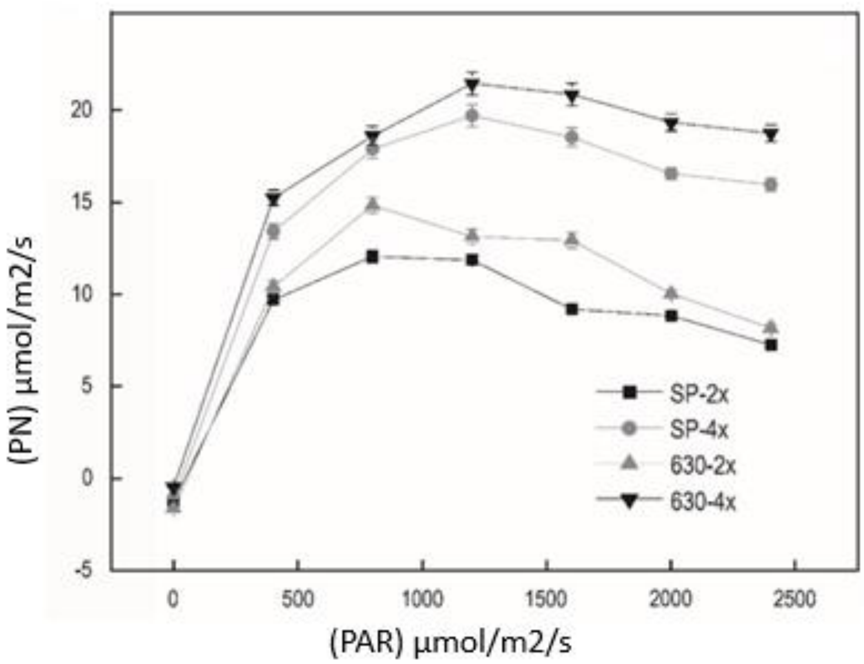
Fig.3. Difference in stomatal size and number between diploid and tetraploid rice (a) Adaxial stomata in control plantlet (b) Adaxial stomata in tetraploid plantlet.

In Figure 3, under a 40x zooming microscope, diploid and tetraploid rice were observed, and it was found that the size of the stomata increased in tetraploid rice compared to normal diploid rice. By observations of those characteristic polyploidy can be confirmed.

3.5 Importance of polyploidy in rice

3.5.1 Greater photosynthetic rate

In a study, it was found that at the late growth stage, autotetraploid rice had a greater photosynthetic rate than diploid rice (Ruan *et al*., 2016). Polyploidy offered the possibility of high rice production as photosynthesis rates increased (Yang *et al*., 2014).



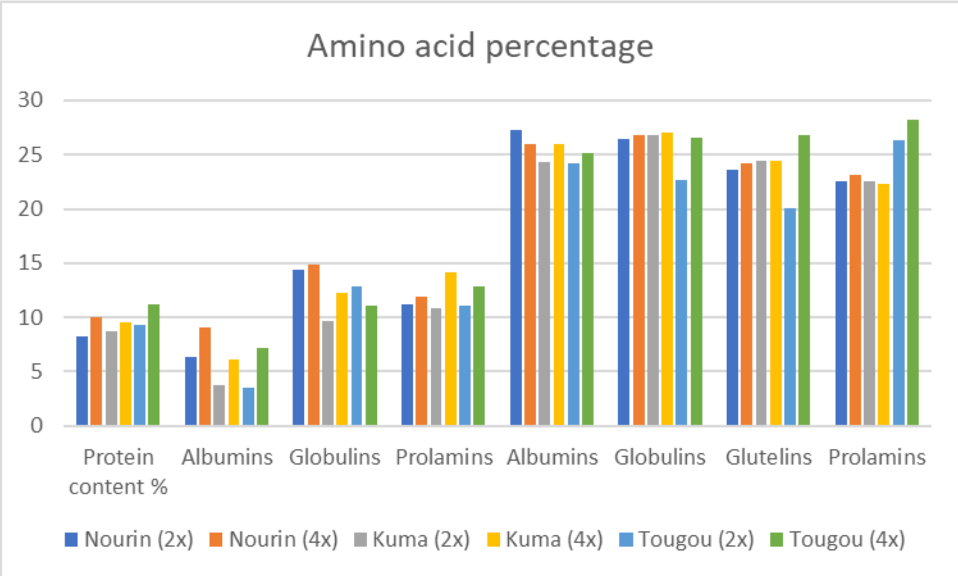
Source: (Yang *et al.*, 2014)

Fig.4. Photosynthetic-light curves of flag leaves in diploid and autotetraploid rice at water potential –40 kPa. (2x) diploid rice line;(4x) autotetraploid rice line, (PAR) photosynthetically active radiation, (PN) Net photosynthetic rate.

From figure 4, it can be seen that at low water potential (40 kPa), if photosynthetically active radiation increases from 0 mol/m2/s to around 1200 mol/m2/s, the net photosynthesis also increases. When photosynthetically active radiation increases by more than 1200 mol/m2/s, plants suffer drought stress and a low net photosynthesis rate, especially diploid rice. In this case, tetraploid rice shows better utilization of solar radiation and has the ability to make more photosynthesis in adverse conditions.

3.5.2 Higher amount of protein and starch and lower amount of amylose content

It was also revealed that compared to diploid rice, auto tetraploid rice has a much higher total amount of protein (xie, 2007). Also the production of starch from photosynthetic products was more effective in autotetraploid rice genotypes than in diploid rice genotypes (Yang *et al.*, 2019). According to a nutritional quality assessment (Song *et al.,* 1993) tetraploid rice has lower amylose content which is desirable in some cases. Due to the fact that autotetraploid glutinous rice has four alleles at the Wx locus, the genotypes of hybrids between autotetraploid glutinous rice and non-glutinous rice are significantly more complex than those of diploid rice (Yang *et al.*, 2019). This has opened a new door for improving rice variety and this helped reveal the starch content in rice. With relation to the utilization of autotetraploid three-line hybrid rice, the methylation modification and Wx gene expression of autotetraploid rice led to important discoveries (Tu *et al.*,2003).



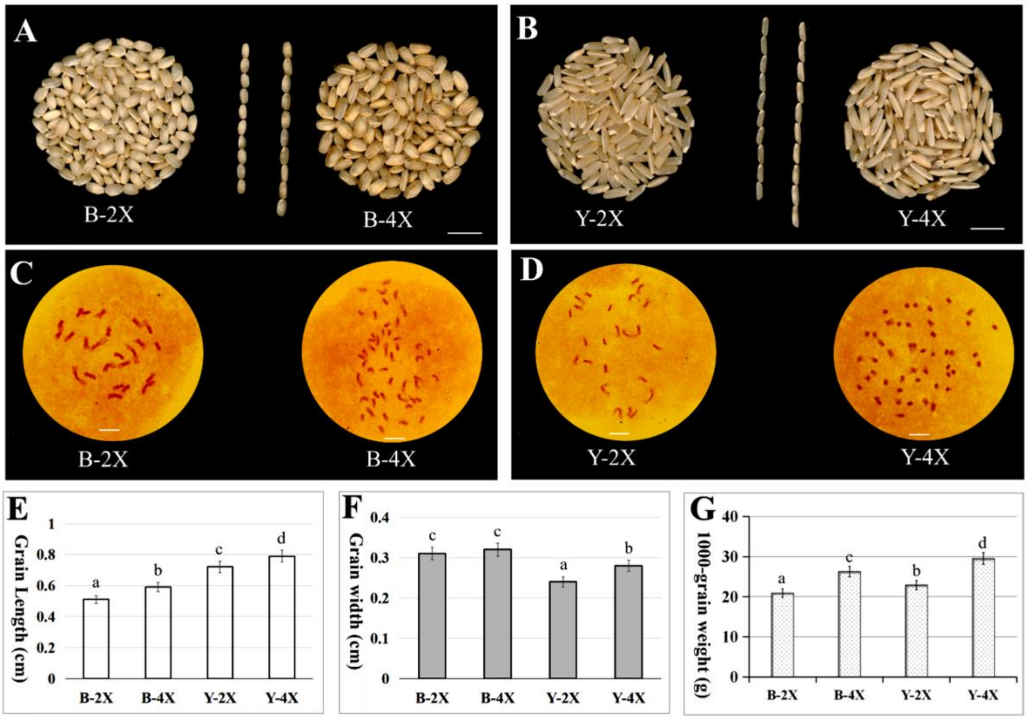
Source: (Arvinder Singh *et al.*, 2010)

Fig.5. Relative contribution of amino acids fractions in diploids and tetraploids of different rice lines.

Figure 5 showed the relative distribution of four protein fractions in the seeds of diploids and tetraploids. The seed protein content in four diploid lines ranged from 8.2% to 9.3%, while their tetraploids had higher protein content ranging from 9.6% to 11.2%. In the case of Nourin, albumin proportions increased from 6.3% in the diploid parent to 9.1% in the tetraploid line. Similarly, the proportion of prolamins increased from 10.9% to 14.1%, 11.1% to 12.9%, and 11.2% to 11.9% in these three tetraploid lines, "Kuma," "Tougou," and "Nourin," respectively.

3.5.3 Increase in length, width and 1000-grain weight

Autotetraploid rice increase in 1000-grain weight, thicker stem, and higher lodging resistance (Cui *et al.*, 2015). Moreover, the tetraploid rice varieties are more nutrient-rich and delicious than the comparative diploid ones.

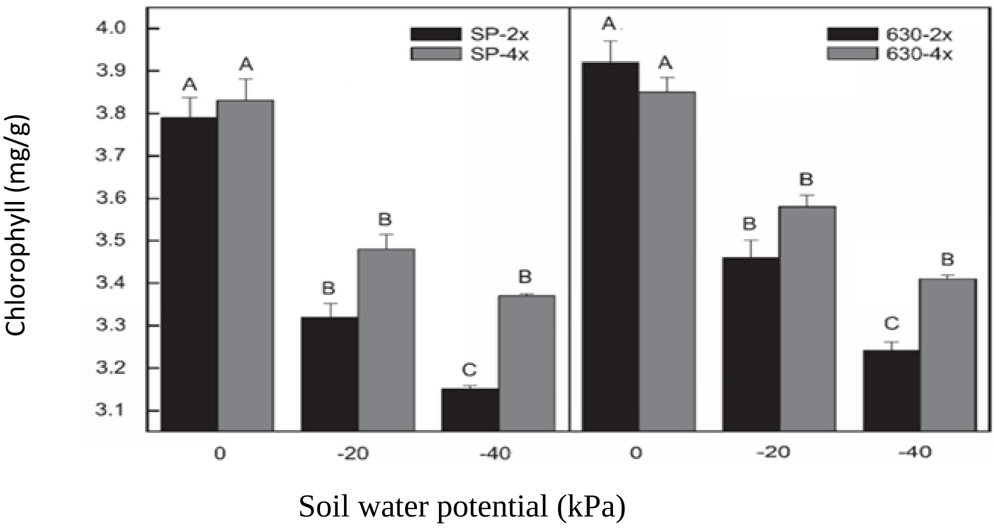


Source: (Wang *et al*., 2022)

Fig.6. Rice grain and root tip chromosome comparison and chromosome identification between diploid and tetraploid rice. (A) Diploid brown rice (B-2X) vs. tetraploid brown rice of (B-4X). (B) Diploid brown rice (Y-2X) vs. tetraploid brown rice (Y-4X (C) B-2X: diploid (2n = 2x = 24); B-4X: tetraploid (2n = 4x = 48). (D) Y-2X: diploid (2n = 2x = 24); Y-4X: tetraploid (2n = 4x = 48). (E–G) Comparison between diploid and tetraploid brown rice: (E) grain length, (F) grain width and (G) 1000-grain weight.

3.5.4 Survival rate is higher under drought condition

Using colchicine and chromosome doubling, it is possible to achieve polyploidy in rice that can survive under drought condition by increasing chlorophyll content (Yang *et al*., 2014). Tetraploid rice can easily biosynthesize jasmonate, which is stress-responsive and has the ability to survive better than diploid rice in saline conditions. This was accomplished through hypomethylation and hypermethylation processes in autotetraploid rice (Wang *et al*., 2021).



Source: (Yang *et al*., 2014)

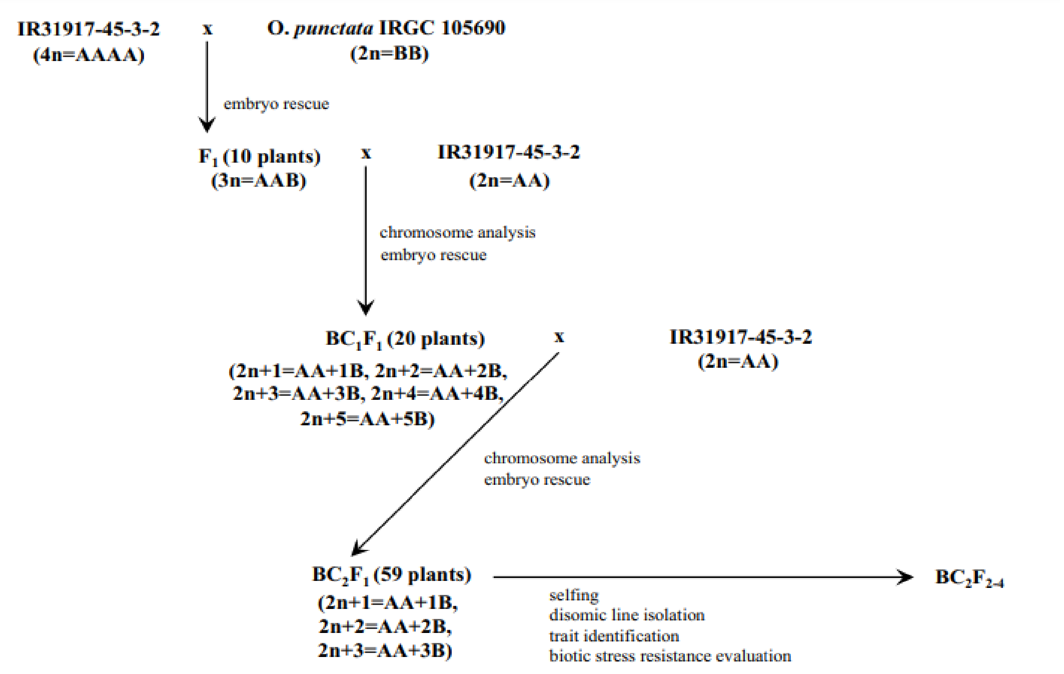
Fig.7. Chlorophyll content in flag leaves of diploid and autotetraploid rice under different severity of drought stress. (2x) diploid rice line; (4x) autotetraploid rice line.

From the above figure 7, it can be seen that under drought conditions, when water potential decreases from 0 kPa to -40 kPa, chlorophyll content also decreases. But autotetraploid rice (4x) has more chlorophyll content in both cases compared to diploid rice (2x), which means autotetraploid rice has a much greater ability to survive under stress conditions by increasing its chlorophyll content.

3.5.5 Distant hybridization is possible through autotetraploid rice

A distant hybridization crossing between wheat and rice resulted in the creation of a new rice autotetraploid genotype (Chen *et al*., 2000). This distant hybridization is very promising for transferring new genes from other species to rice. For instance, the reasons for autotetraploid rice's decreased seed germination and fertility as well as how it reproduces and its interspecific hybrids were described by those interspecific hybrids (Zhang *et al*., 2013).

Through a cross between autotetraploid indica rice and *Oryza punctata*, valuable traits for rice improvement were transferred (Jena *et al*., 2016).



Source: (Jena *et al*., 2016)

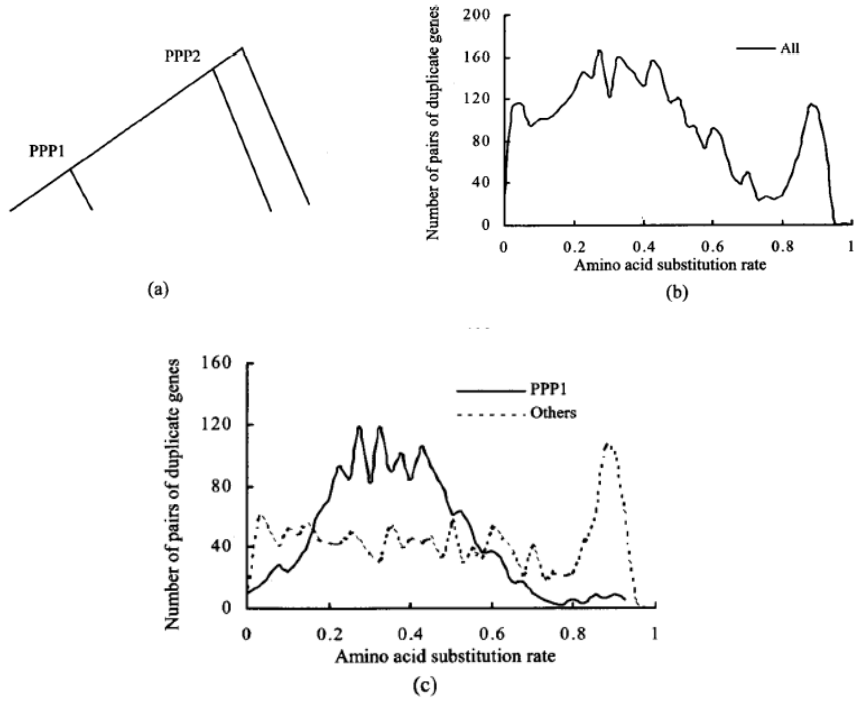
Fig.8. Schematic diagram of the crossing procedure for the production of *O. punctata* **chromosome addition lines using autotetraploid rice line.**

IR31917-45-3-2, a tetraploid rice, was crossed with the diploid IRGC 105690 accession of O. punctata. They produce F1 with a triploid genotype, and F1 was crossed with diploid IR31917-45-3-2 lines (which were used as a parent to produce tetraploid). After the cross, 20 plants of BC1F1 were produced. Those progenies were backcrossed again with diploid IR31917-45-3-2 lines. After successful backcrossing, stable genotypes were selfed, and they produced a disomic line with biotic stress resistance capacity.

Transgenics through sophisticated methods is very costly and laborious. Because of the genetic barrier in rice, the transgene method is used to transfer any essential gene from another species. For instance, when the OsSGL gene was added to a rice genotype, transgenic rice plants exhibited a variety of beneficial features, such as increased grain length, grain weight, and grain quantity per panicle, which led to a notable increase in yield (Wang *et al*., 2016). Autotetraploid rice can undergo such types of distant hybridization, which can be cost-effective and easily desired genes can be transferred.

3.5.6 Polyploidy in rice has importance in the evaluation process

Polyploidy in rice also has importance in the evaluation process. In nature, it was found by using a molecular clock that two rice ancestors were derived with polyploidy million years ago (Zhang *et al*., 2005).



Source: (Zhang *et al*., 2005)

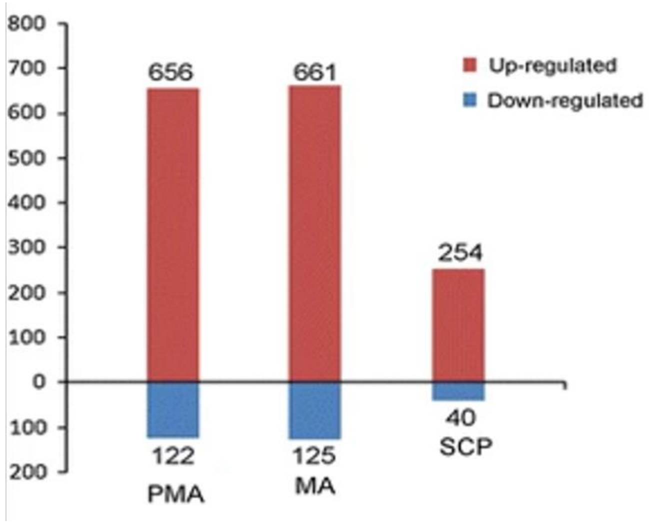
Fig.9. Evolutionary history of polyploid rice. (4a): a whole-genome duplication of (PPP1 and PPP2) for the rice genome (4b): frequency distribution of pairs of duplicate genes on duplicate blocks of PPP1 4(c): PPP 1 caused the first peaks but the second peak of the PPP2 distribution was 0.875, indicating that the ancient polyploidy event happened approximately 220 million years ago.

Through an evolutionary process of adjusting chromosomal number, increasing genome size, tandem repeats of DNA sequences, genes, regulatory sequences of genes, and their transcription and translation processes, it is possible to produce novel genotypes that may help an organism survive in stressful environments (Heslop *et al*., 2022).

3.6 Challenges of autotetraploid rice

3.6.1 Abnormal pollen development in autotetraploid Rice

The commercial utility of autotetraploid is limited due to their pollen sterility. In autotetraploid rice, polyploidy amplifies the epistatic effect, resulting in variable gene expression patterns and higher pollen sterility.



Source: (Wu *et al*., 2015)

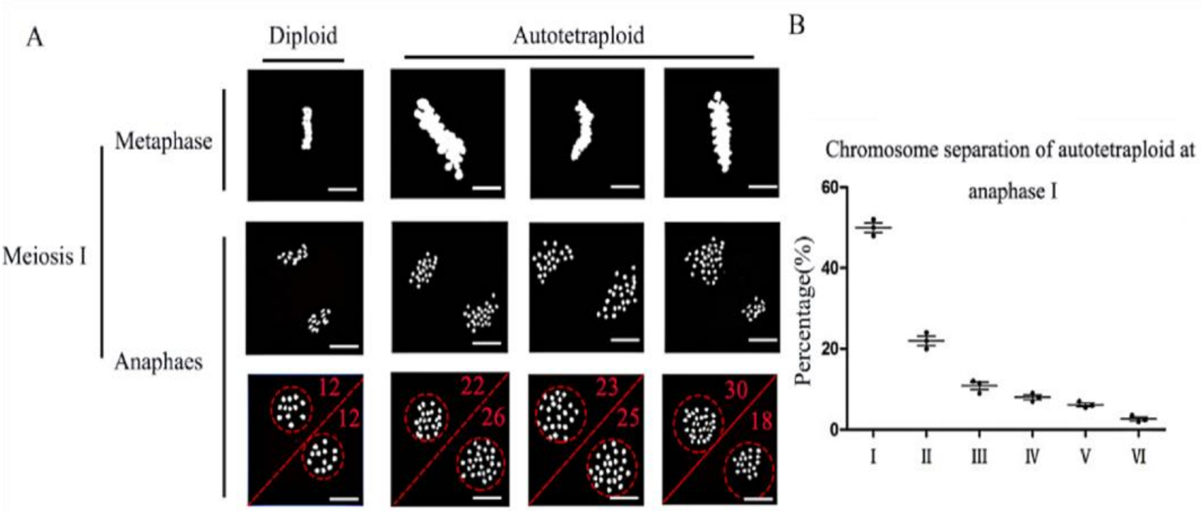
Fig.10. Identification of the expressed genes during the pollen development stage. (PMA) Pre-meiotic interphase, (MA) Meiotic, (SCP) Single microspore stage in autotetraploid rice.

According to the figure 10, 778 genes were differentially expressed in the pre-meiotic interphase of autotetraploid rice, with 656 and 122 being up- and down-regulated, respectively. A total of 786 genes were found to be differently expressed during meiosis. 294 genes were found to be differently expressed during the microspore stage. Down-regulated genes hinder cellular prolification and appropriate pollen formation, while up-regulated genes promote it. In diploid upregulated genes are more in number than autotetraploid rice that’s why diploid rice develops normally than autotetraploid rice (Wu *et al*., 2015).

It was observed that embryo sac and pollen fertility are major factors determining the seed setting rate of autotetraploid rice (Shahid *et al*., 2010). The EAT1 gene encodes a transcription factor that controls the expression of genes involved in tapetum development, which is required for pollen formation. Male sterility can result from abnormal gene regulation in polyploid rice (Kamara *et al*., 2022). The gene OsABCG15 encodes a membrane transporter involved in lipid transport to the anther, which is essential for pollen formation (Lu *et al*., 2020). Polyploid rice pollen sterility can also be caused by mutations in this gene.

3.6.2 Abnormal meiosis in autotetraploid rice:

Homologous chromosomes must pair and synapse appropriately during meiosis to achieve normal chromosomal segregation in gametes. There are four sets of chromosomes in autotetraploid rice, which may make pairing and segregation more difficult, leading to defects such as non-homologous chromosome pairing, univalent formation, and multivalent formation (Svačina *et al*., 2020).



Source: (Ku *et al*., 2022)

Fig.11. Impaired meiosis in autotetraploid rice. (A) Meiosis of autotetraploid rice with abnormal chromosome segregation behaviour (B) Chromosomal segregation percentage at the meiotic stage.

In Figure 11, it is seen that diploid rice genotype chromosomes segregate equally during meiosis. But in the case of autotetraploid rice chromosomes, they don't segregate equally all the time. That's why aneuploid plants and animals become sterile. Mutations in genes involved in meiotic processes can also result in improper meiosis in autotetraploid rice. Mutations in genes involved in DNA repair, chromosomal segregation, or spindle formation which can cause abnormal meiosis and low fertility or sterility in autotetraploid rice (Ku *et al*., 2022).

3.6.3 Genetic instability in autotetraploid rice:

Some quantitative equilibrium among the genes may be disrupted when a diploid variety of rice with normal fertility is changed to an autotetraploid variety with poor fertility. For example, it may assume that the gene combination (a+ a+ b+ b+) is beneficial for fertility in diploids, but its quadruple state (a+ a+ a+ a+ b+ b+) may be unfavourable because of the differing dosage effects of different unfavourable genes. The partial recovery of fertility in autotetraploid rice may lead to monohybrid heterosis, which makes it very difficult to produce stable autotetraploid rice genotypes. (International Rice Research Institute, 1986).

3.7 Prospects for autotetraploid rice in the future

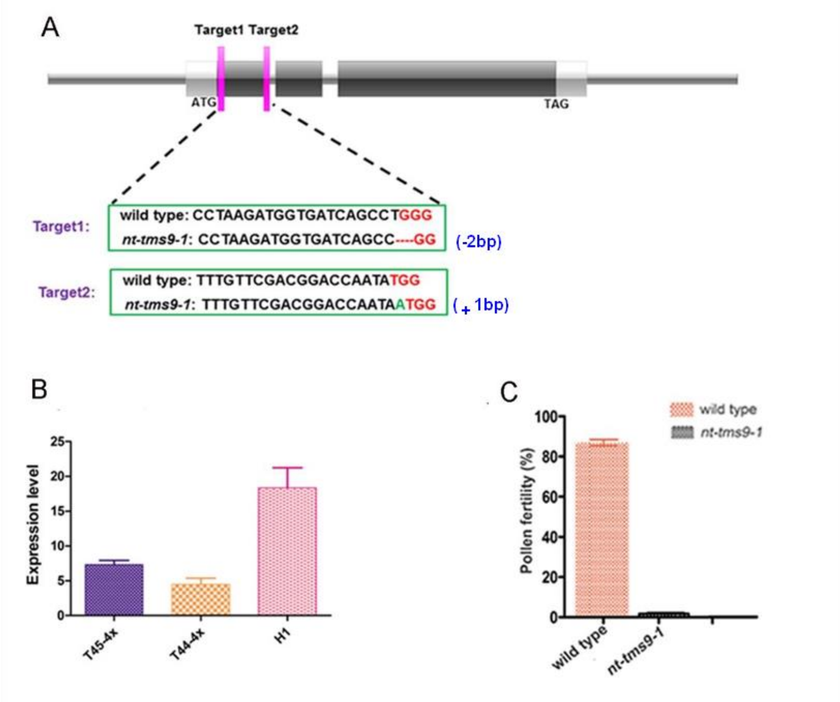
Normal seed set is also a serious problem in autotetraploid rice. OsMND1 has a key function in regulating meiosis, enhancing pollen fertility, and minimizing early embryo abortions. It also influences the regulation of some critical meiosis-related genes, resulting in an increase in seed set rate (Xiong *et al*., 2019). Modification of this gene in polyploid rice so that it can perform well can be a solution to restore seed set problems.

Rice polyploidization is an ever-changing process (Wang *et al*., 2005). Artificial production of polyploidy has resulted in severe seed sterility, but fertile autotetraploids, known as the Polyploid Meiosis Stability and Neo-Tetraploid lines, have recently been discovered with a recovery of seed fertility (Koide *et al*., 2020), which is promising for future rice improvement.

Genetic male sterile polyploid rice lines that are thermo and photosensitive supply resources for future research into polyploidy and hybrid vigour, and enhance polyploid hybrid rice for further exploitation (Zhang *et al*., 2017).

Nutritional issues are a major issue all around the world. Variations in rice nutritional quality as a result of polyploidization may provide a new scientific base for breeding elite nutrient-rich rice varieties through polyploidization (Wang *et al*., 2022).

Pollen fertility is a great concern in case of autotetraploid rice. TMS9-1, TMS5, and other pollen fertility-related genes have been modified using the CRISPR/Cas9 technique (Wu *et al*., 2020) paving the way for autotetraploid rice improvement.



Source: (Wu *et al*., 2020)

Fig.12. Mutations of TMS9-1 target sites and its expression level in H1 and nt-tms9-1. (A) PAM sequence and two target sites of TMS9-1 in neo-tetraploid rice (B) Expression level of TMS9-1 in neo-tetraploid rice and its two parents (C) Pollen fertility of TMS9-1 knockout lines (nt-tms9-1) and its wild type.

TMS9-1 is important for the restoration of pollen fertility. From figure 12 it can be seen that when TMS9-1 undergoes mutation, such as in a polyploid state, it may express nt-TMS9-1 gene expression, which leads to pollen sterility. Whenever the TMS9-1 gene was knocked out, the nt-TMS9-1 gene expressed itself, and one line of polyploid rice showed 100% pollen sterility compared to its wild type, which has the TMS9-1 gene. This modification can be a new way to improve fertility in polyploid rice.

CHAPTER 4

CONCLUSION

To sum up, while there are different methods of inducing polyploidy in rice, such as soaking rice seeds in colchicine, splitting the application of colchicine in germinated coleoptiles, and applying colchicine to rice callus, the most effective method has been found to be the application of colchicine to rice calluses because the success rate of this method ranges from 31% to 65.5%, while the other two methods are below 45%. This callus method allows for better control and a higher success rate of polyploid induction, making it a promising technique for improving crop yield and other desirable traits in rice breeding programs.

Polyploidy in rice is important because it enhances the crop's ability to withstand environmental stress caused by climate change. It also increases the genetic diversity of rice germplasm, which is crucial for developing new and improved varieties. Polyploidy can also improve the photosynthetic capacity and increase grain size, yield. When photosynthetically active radiation reach 1200μmol/m^2/s above optimum level polyploid rice can perform more better than diploid rice, protein content of polyploid rice ranging from 9.6% to 11.2% which is more compare to diploid rice make it more nutritious. Additionally, it allows for distant hybridization, which can lead to the development of novel rice varieties with desirable traits.

To improve barriers of polyploidy in rice, it is important to address issues such as abnormal pollen development, genetic instability and abnormal meiosis. New gene introgression, which have the possibility to make stable genotypes such as manipulation of the nt-TMS9-1 gene helps restore pollen fertility. Furthermore, proper colchicine application techniques with high efficacy can result in the development of new and promising rice genotypes for future aspects.

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