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FULL PAPER

Determination of Optimal Panicle Harvest Timing and Evaluation of Anther Culture Response In Turkish Rice Varieties for Haploid Breeding

Gihwan Yi[1,2*], Soon Ki Park[3], and Myung-Hee Kim[4]

[1]Department of farm management, Kyungpook National University, 80 Daehakro 41566, Republic of Korea; [2]Institute of Agricultural Science & Technology, Kyungpook National University, 80 Daehakro 41566, Republic of Korea; [3]School of Applied Biosciences, Kyungpook National University, 80 Daehakro, Republic of Korea; [4]Genomics Division, Department of Agricultural Bio-Resources, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 54874, Republic of Korea.
*Corresponding author, gihwan@knu.ac.kr

This study aimed to evaluate the anther culture response in Turkish rice varieties and assess the feasibility of introducing haploid technology into rice breeding programs in Turkey. Histological analysis revealed that when the anther tip was located between one-third and half of the spikelet' length, about 96% of the pollen was in the uninucleate stage. When the anther tip was positioned between half to three-fourths of the spikelet' length, most of the pollen was in the tricellular stage. The optimal time to harvest panicles for anther culture is when the anther tip located between one-third and one-half of the spikelet's length, with the position closer to one-third being more favorable. The callus induction rate among 39 rice varieties ranged from 0.1% to 48.9%, with an average of 9.9%. The green plant regeneration rate varied 0% to 61.1%, with an average of 10.0%, while green-to-albino ratio was 0.3. The highest rates of both callus induction and plant regeneration were observed in the varieties 'Surek 95' with rates of 48.9% and 61.1%, respectively. The primary challenges in the anther culture of Turkish rice varieties were the risk of contamination during *in vitro* culture and the high frequency of albino plant occurrence. In conclusion, although differences in anther culture response were observed among the varieties, the introduction of haploid techniques into Turkish rice breeding programs appears feasible on a limited scale.

Keywords: Anther, culture, response, rice, Turkey

INTRODUCTION

Turkey launched its rice research program in 1970, and a systematic breeding program was started in 1982 under the guidance of the National Rice Research Projects (Sürek 1997). The Thrace Agricultural Research Institute (TARI) in Edirne is mainly responsible for coordinating rice breeding programs. Over the past three decades, the Turkish rice sector demonstrated significant potential to establish Turkey as a major rice producer in the Eurasian region. The rice yield has increased from 4 t ha^{-1} in 1970 to 9 t ha^{-1} in the 2010s (USDA 2025). Since 1990, when the first Turkish rice varieties were developed, 63 varieties have been bred and registered in the National Catalog of Turkey (VRSCC 2019). In the past 30 years, there have been huge advances in rice varietal improvement in Turkey. However, the Turkish rice sector continues to face numerous challenges, including abiotic and biotic stresses caused by climate change (Surek 1997). Low genetic diversity could become a significant constraint in future rice breeding of Turkish varieties. Additionally, Turkish rice has a longer maturation period, a shorter caryopsis, and lower head rice recovery compared to Italian varieties (Surek and Yi 2020). To address these issues, the early development of rice varieties that meet current demands is essential.

Rice breeding in Turkey is primarily based on the pedigree method. While this method is a reliable approach for breeding self-pollinating crops, it is time-consuming and inefficient for selecting early generations, espacially under direct seeding conditions. Anther culture is a haploid breeding technique in which haploid plants are regenerated by *in vitro* culture of immature anthers. Homozygous doubled haploid (DH) plants are rapidly obtained by the chromosome doubling of haploid plants.

Unlike the pedigree breeding method, which requires seven to eight generations to achieve homozygosity, DH plants can be developed in just one generation (Morrison and Evans 1987; Taskin and Bilgili 2023). DHs techniques offer many benefits to plant breeders by shortening the breeding cycle and providing a highly effective means of selecting for recessive agronomic traits compared to traditional pedigree method.

Turkey and Europe primarily practice direct-seeded rice cultivation, with weedy rice being one of the major challenges. To address this issue, Clearfield® technology, which utilizes the imidazolinone-resistant gene (IMI-R), has been widely adopted (Singh et al. 2017). Since 2009, a total of 34 Clearfield® rice varieties have been registered in the EU Plant Variety Database. In Italy, Clearfield® rice accounts for 35% of the total rice cultivation area (Hansjoerg 2017). Anther culture is expected to be highly effective for introducing the IMI-R trait into existing varieties, as it shortens the breeding period and improves selection efficiency compared to traditional pedigree breeding methods. However, there has been little research on anther culture or its efficiency for breeding varieties in Turkey. Moreover, DH technology supplies genetic material for the exploitation of quantitative trait loci (QTLs) and genes associated with various agronomic traits. This experiment aimed to evaluate the response of Turkish rice varieties to anther culture and investigate the optimal timing for panicle harvest suitable for culture. Additionally, several challenges were analyzed to facilitate the introduction of anther culture into Turkish rice breeding programs.

MATERIALS AND METHODS

Determination of the optimal timing for panicle harvest suitable for anther culture.

The Turkish variety ‘Osmancik-97’, the Italian variety ‘Baldo’, and the Korean variety ‘Baekilmi’ were used to determine the optimal timing for panicle harvest suitable for anther culture. The developmental stages were categorized based on the position of the anther tip within the spikelet: (1) half to one-third the length of the spikelet (Figure 1A) and (2) half to three-fourths the length of the spikelet (Figure 1B). The anthers from each position were harvested and placed in a fixative solution consisting of ethanol and acetic acid in a 3:1 ratio for 2 hrs. The fixed samples were washed with 100 mM Tris-HCl (pH 7.5) to remove impurities. The samples were then stained using a DAPI staining kit (GenScript Corporation) to determine the developmental stages of the microspores. The staining solution was a mixture of 20% methanol and 1 µg of DAPI (4′,6-diamidino-2-phenylindole). We fixed in a 3:1 (v:v) solution of ethanol and acetic acid prior to staining with DAPI. After incubation for 1.5 hrs at 60°C, the samples were examined under a fluorescence microscope (Figure 2). Anthers in DAPI solution on the microscope slides were then opened with dissecting needles and gently squashed under a cover slip. Images were captured from the ProgRes MFcool camera for investigating DAPI epifluorescence under a Nikon ECLIPSE 80i microscope (Nikon).

Anther Culture

Thirty-nine rice varieties bred in Turkey between 1990 and 2019 were transplanted on May 27, 2023, into the rice breeding field of Kyungpook National University, Korea. The rice varieties used in this experiment was provided by Dr. Surek of the Trakya Agricultural Research Institute (TARI), Edirne, Turkey.These varieties were cultivated following standard rice-growing protocols. At the booting stage, panicles were harvested by identifying the position of the anther tip within the spikelet. Immediately after collection, the panicles were placed in a cold chamber (Conviron; www.conviron.com) at 12°C 1°C for 15 days. During this cold pretreatment period, moisture was maintained using a bottle with dimensions of 10–15 cm in diameter and 25 cm in height. with moisture supply using a bottle (diameter, 10–15 cm; height, 25 cm) for cold pretreatment (Chung and Sohn 1986).

The anther culture was performed following the standard protocols for rice anther culture recommended by the National Institute of Crop Science, Rural Development Administration, Korea (Yi et al. 2003). The modified N_6 medium (N_6-Y_1, Chung 1985) was used as a basal medium for callus induction and plant regeneration. The cold-treated spikelets were surface-sterilized with 70% ethanol and then air-

Figure 1. The position of the anther tip in the spike (A: half to one-third length; B: half to three-fourth length).

dried to remove excessive moisture in the cleanbench. After drying, the basal part of spikelet was cut off using scissors. Using tweezers, the cut spikelets were picked up and tapped against the wall of the petri dish (Falcon 1007) to deposit anthers onto the medium. For callus induction, the medium was supplemented with 2 ppm NAA, 0.5 ppm KI, and 0.2% casein hydrolysate as plant growth regulators. Additionally, 2% sucrose and 3% sorbitol were used as carbon sources, and 0.5% Phytagel (Sigma P8169) was used as the gelling agent. The rate of callus induction was calculated as the proportion of anthers that developed calli after 30 days of incubation at 25°C ± 1°C under dark conditions. For plant regeneration, the medium was supplemented with 2 ppm KI, 0.2 ppm IAA, 2% sucrose, 3% sorbitol, and 0.5% Gelrite. The regeneration process was conducted over 30 days at 25°C ± 1°C under a light intensity of 2,500 lux, with a 14-hr light/10-hr dark cycle (Yi et al. 2003). The plant regeneration rate was determined by dividing the number of green plantlets with both shoots and roots by the number of anthers from which calli were successfully induced. The callus induction rate and plant regeneration rate were evaluated for each Petri dish, and the average and standard deviation were calculated.

RESULTS AND DISCCUSION

Developmental stages of pollen microspore

The developmental stages of pollen microspore were investigated based on the position of the anther tip within the spikelet, determining the optimal developmental stages of pollen for anther culture in European and Korean rice varieties (Table 1). ‘Osmancik-95’ is a leading rice variety in Turkey, while

Table 1. Stages of pollen microspore development determined by the position of the anther tip within the spikelet.

Variety	Position	Uninuclear (%)	Bicellular (%)	Tricellular (%)	Total (%)
Osmancik-97	⅓~½	168 (96.0)	7 (4.0)	0 (0.0)	175 (100)
	½~¾	0 (0.0)	0 (0.0)	264 (100)	264 (100)
Baldo	⅓~½	215 (96.4)	8 (3.6)	0 (0.0)	223 (100)
	½~¾	0 (0.0)	2 (0.7)	278 (99.3)	280 (100)
Baekilmi	⅓~½	214 (99.1)	2 (0.9)	0 (0.0)	216 (100)
	½~¾	0 (0.0)	9 (3.8)	230 (96.2)	239 (100)

Table 2. Callus induction rates according to the position of the anther tip within the spikelet.

Cultivar	Position of anther tip	No. of anther		Callus induction (Mean±SD, %)
		Sum	Inoculated/PD (Mean±SD)	
Osmancik-97	⅓	1,337	44.6±6.7	468 (35.0±13.7)
	½	1,724	66.3±7.6	471 (26.7±15.6)
	⅔	2,939	69.6±7.7	29 (1.5±2.6)
Nipponbare	⅓	1,561	52.1±5.5	260 (16.8±7.0)
	½	1,229	58.5±6.5	136 (11.1±5.6)
	⅔	1,146	60.3±6.1	4 (0.4±0.7)

Table 3. Distribution of callus induction and green& albino plant regeneration rates in anther culture of Turkish rice varieties.

Range(%)	Name of variety	Plant regeneration	
	Callus induction	Green	Albino
~10	Altinyazi, Beser, Demir, Edirne, Ergene, Gala, Gonen, Halilbey, Hamzadere, Ipsala, Kale, Karadeniz, Kiral, Kirkpinar, Kizilirmak, Kuplu, Manyas Yildizi, Meric, Mevlutbey, Negis, Pasali, Şumnu, Tosya Gunesi, Trakya, Yavuz	Biga Incisi, Cakmak, Demir, Ece, Edirne, Efe, Efsane CL, Ergene, Halilbey, Hamzadere, Haziran, Karadeniz, Kargi, Kiral, Kizilirmak, Kuplu, Manyas Yildizi, Meric, Mevlutbey, Negis, Osmancik-97, Pasali, Sumnu, Surek M1711, Tosya Gunesi, Tunca, Ulfet, Yavuz	Beser, Cakmak, Ece, Efe, Efsane CL, Ergene, Halilbey, Hamzadere, Kiral, Manyas Yildizi, Tosya Gunesi, Trakya
10~20	Bafra Yildizi, Biga Incisi, Cakmak, Efsane CL, Haziran, Surek M1711, Tunca, Ulfet, Yatkin	Bafra Yildizi, Gonen, Kirkpinar, Trakya, Yatkin	Altinyazi, Biga Incisi, Edirne, Gonen, Karadeniz, Negis, Osmancik-97, Sumnu, Tunca, Ulfet, Yavuz
20~30	Kargi, Ece, Efe,	Altinyazi, Beser, Gala, Ipsala, Kale	Bafra Yildizi, Haziran, Kargi, Kirkpinar, Kuplu, Surek M1711
30~	Osmancik-97, Surek 95	Surek 95	Demir, Gala, Ipsala, Kale, Kizilirmak, Meric, Mevlutbey, Pasali, Surek 95, Yatkin

'Baldo' represents a key variety from Italy. 'Baekilmi', an early-maturing variety from Korea, exhibits agronomic traits similar to Osmancik-95. In the Turkish variety 'Osmancik-97' and Italian rice 'Baldo,' when the anther tip was positioned between halfway to one-third in the spikelet's length (Figure 1A), ~96% of the pollen was uninucleate, with the remaining 4% being binucleate. In contrast, in the 'Baekilmi', 99% of the pollen was uninucleate (Figure 2 stage 1). When the anther tip was positioned between half to three-fourths of the spikelet's length (Figure 1B), none of the varieties exhibited uninucleate pollen. However, 3.8% of the pollen from 'Baekilmi' and 0.7% from 'Baldo' were binucleate. Furthermore, 96.2% of the pollen from 'Baekilmi' and nearly 100% from 'Osmancik-97' and 'Baldo' were tricellular (Figure 2 stage 2). However, there appears to be a slight delay in pollen developmental stages in the 'Baekilmi' compared to 'Baldo'. These findings suggest that the developmental stages of pollen in European and Korean rice were not significantly different based on the positioning of the anther tip within the spikelet. The position of the anther tip within the spikelet is used as a simple indicator for determining the developmental stage of pollen, and it is believed that this method can also be applied to Turkish rice varieties. (Chung and Sohn 1986). Previous studies have indicated that the developmental stage of the pollen influences the frequency of callus induction. In *japonica* rice, the early to mid-uninucleate stages (Chung and Sohn, 1986) and in *indica* rice, the uninucleate to early binucleate stages were favorable for anther culture (Cho and Zapata 1990).

Callus induction based on location of anther tip within the spiklet

To investigate callus induction based on the location of the anther tip within the spikelet, the leading Turkish variety 'Osmancik-97' and the typical Asian japonica variety 'Nipponbare' were selected. The callus induction rate varied depending on the position of the anther tip within the spikelet (Table 2). When the anther tip was positioned in the first third length of the spikelet (Figure 3A), the callus induction rate was 35.0% in 'Osmancik-97' and 16.8% in the reference variety 'Nipponbare'. When the anther tip was positioned in the second half length (Figure 3B), the rates were 26.7% and 11.1%, respectively. However, when it was positioned in the second third of the spikelet length (Figure 3C), the rates dropped significantly to 1.5% and 0.4%, respectively.

Afza et al. (2000) report that the callus-forming ability of anthers was the highest at the basal part and the lowest at the top within the same panicles. Afza et al. (2000) reported that the callus-forming ability of anthers was highest at the basal part and when containing pollen at the uninucleate and early-middle

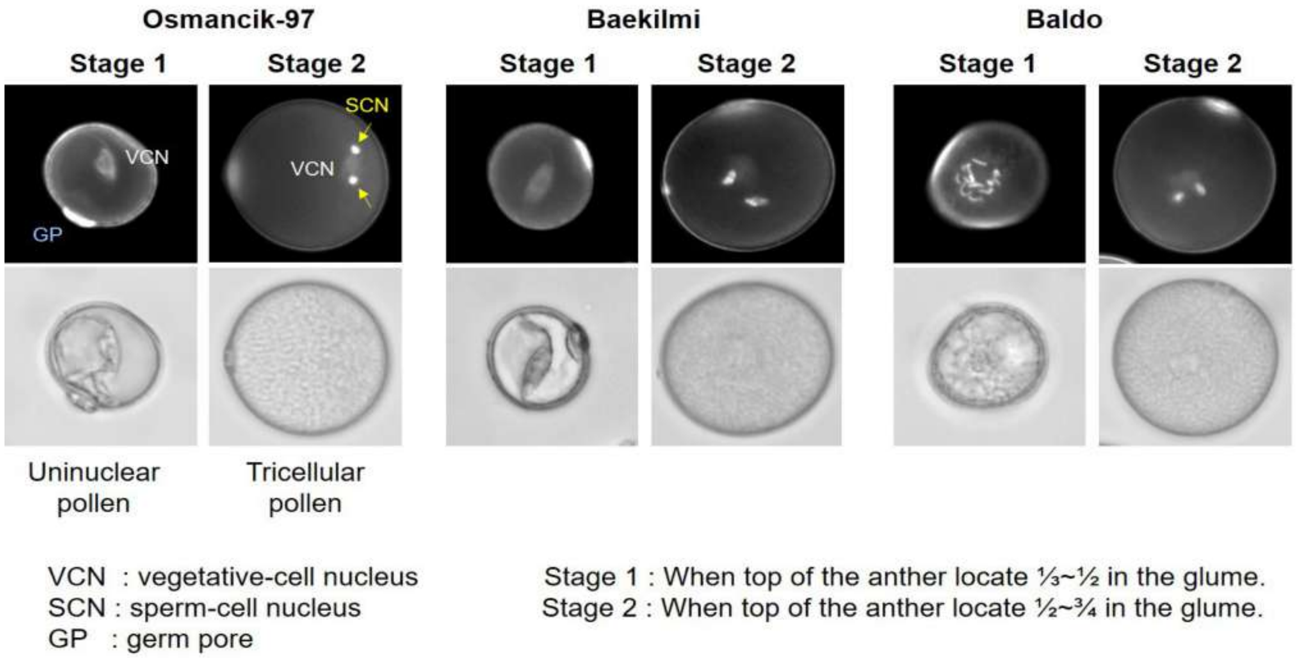

Figure 2. DAPI staining of the vegetative-cell nuclei and sperm-cell nuclei at various pollen microspore developmental stages in rice.

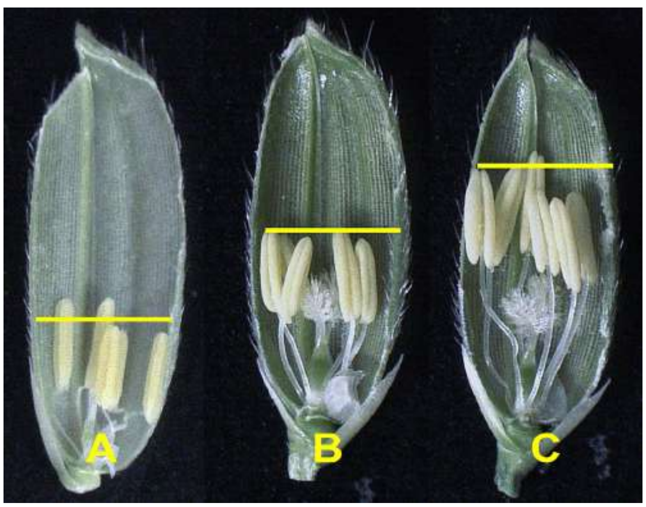

Figure 3. Positions of anther tips within the spikelet: A) first third, B) second half, C) second third.

stages, compared to those from the middle and top parts. The optimal position for callus induction in Turkish rice varieties was found to be between one-third and one-half of the spikelet length, with enhanced favorability observed as it approached the one-third length position (Table 2).

Anther culture responses in Turkish rice varieties

The callus induction rates in anther culture varied among Turkish rice varieties, ranging from 0.1% to 48.9%, with an average rate of 9.9% (Table 3, 4). Notably, 'Surek 95' exhibited the highest rate of callus induction at 48.6%, while 'Efe', 'Ece', and 'Kargi' displayed rates of 29.2%, 26.5%, and 21.2%, respectively. Conversely, 'Ergene', 'Kirkpinar', 'Manyas Yildizi', 'Meric', and 'Yavuz' demonstrated callus induction rates below 1.0%. The callus induction rates for the japonica reference cultivars, 'Baekilmi' and 'Nipponbare', were 9.3% and 16.8%, respectively.

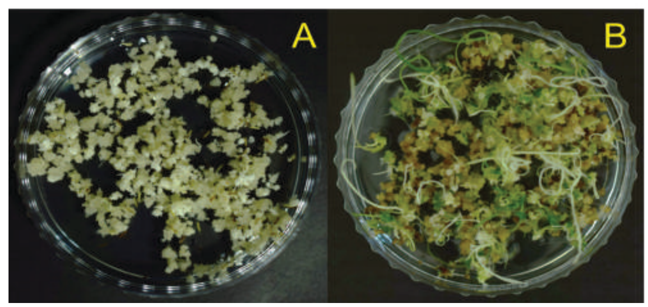

Figure 4. Callus induction and plant regeneration of ‘Surek 95’.

The regeneration rates of green and albino plants in anther culture varied among Turkish rice varieties, ranging from 0% to 61.1% and 1.4% to 76.6%, respectively (Table 3, 5). On average, the regeneration rate of green plants was 10.0%. Remarkably, 'Surek 95', which exhibited the highest callus induction rate, also demonstrated the highest regeneration rates of green and albino plants at 61.1% and 76.6%, respectively. Additionally, the green plant regeneration rates in 'Altinyazi', 'Beser', 'Gala', 'Ipsala', and 'Kale' exceeded 20%. Moreover, the albino plant regeneration rates in 'Demir', 'Gala', 'Kale', 'Kizilirmak', 'Meric', 'Mevlutbey', 'Pasali' and ‘Surek 95’ were greater than 40%. The average albino plant emergence ratio was 0.3, ranging from 0.1 to 10.7. ‘Beser’ exhibited the highest green to albino plant regeneration ratio (10.7), followed by ‘Trakya’ (2.2), ‘Efsane’ (1.5), ‘Chakmak’ (1.3), and ‘Ece’ (1.2). In contrast, ‘Demir’, ‘Haziran’, ‘Kargi’, ‘Melutbey’, and ‘Osmancik-97’ had the highest albino plant regeneration ratio, each being less than 0.2. The plant regeneration rates in the reference cultivars, 'Baekilmi' and 'Nipponbare,' were 4.2% and 34.2%, respectively, with a green-to-albino plant regeneration ratio of 0.4 and 3.0, respectively (Table 3, 5). The variety with the highest callus induction and plant regeneration rates

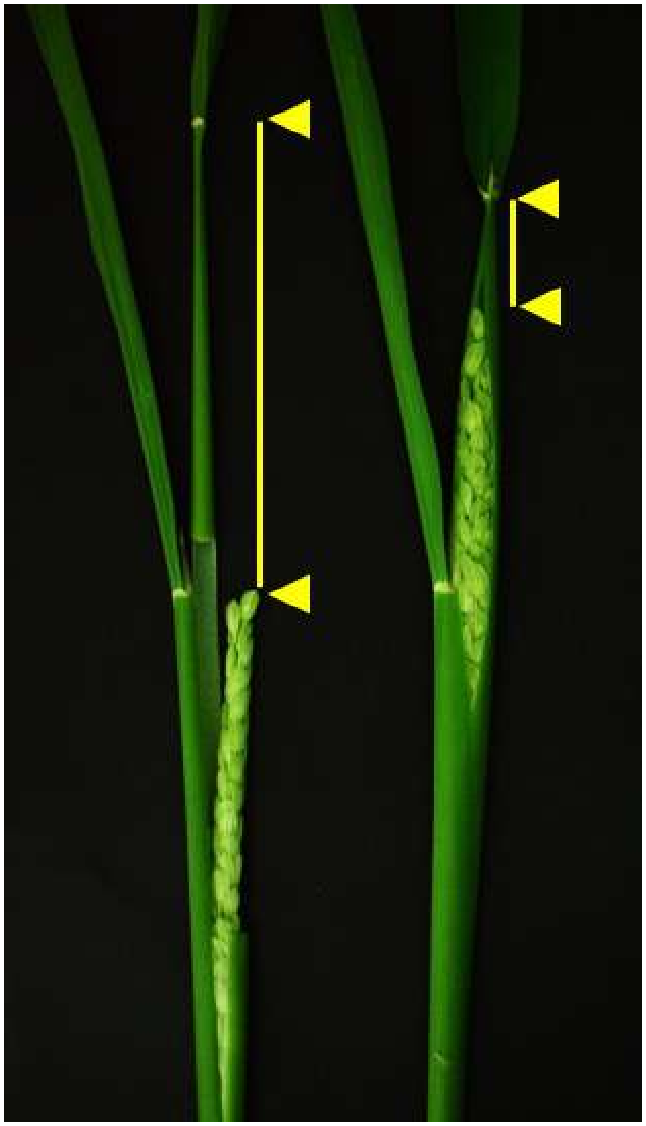

Figure 5. Positions of anther tips within the spikelet: A) first third, B) second half, C) second third.

was 'Surek 95,' with rates of 48.9% and 61.1%, respectively (Figure 4).

'Surek 95' is a high-yielding variety registered in 1995, developed through the crossbreeding of the Italian varieties 'Roca' and 'Rodina'. Further genetic analysis of its the high regeneration rate should be explored in future studies. 'Surek 95' holds promise for exploring genes and quantitative trait loci (QTLs) associated with callus induction and plant regeneration in rice anther culture. Furthermore, it may offer valuable resources for genetic and cytological studies on in vitro organ differentiation. While reference data on anther culture responses in Turkish rice varieties is limited, significant variations exist among the varieties. According to He et al. (2006), *japonica* varieties generally exhibit superior anther culture responses compared to *indica* varieties. DNA analysis has shown that the origin of European rice includes tropical *Japonica* found in the United States and Argentina, as well as two groups of temperate *Japonica* (Brigitte et al. 2012). Among 34 of 39 Turkish rice varieties, 34 belonged to the same two groups as Italian varieties (Comertpay et al. 2015) and 62% have at least one Italian parent (Surek and Yi, 2020). Additionally, Camilo et al. (2018) found that *tropical japonica* varieties demonstrate greater responsiveness to callus induction and plant regeneration compared to *Mediterranean japonica* or *indica* varieties.

Table 4. Callus induction in the anther cultures of Turkish rice varieties.

Cultivar	No. of anther		Callus induction (Mean±SD, %)
	Sum	Inoculated/PD (Mean±SD)	
Altinyazi	1,864	64.3±5.9	64 (3.4±3.9)
Bafra Yildizi	1,815	64.8±6.0	278(15.0±8.8)
Beser	2,237	74.6±7.9	116 (5.4±5.2)
Biga Incisi	1,834	65.5±4.4	232 (12.7±7.6)
Cakmak	1,702	60.8±6.6	294 (17.2±11.8)
Demir	2,089	69.6±7.7	201 (9.8±6.7)
Ece	1,981	70.8±6.9	528 (26.5±12.7)
Edirne	1,637	68.2±8.2	57 (3.4±3.7)
Efe	1,902	63.4±6.0	553 (29.2±12.0)
Efsane CL	1,867	66.7±6.0	201 (11.2±7.6)
Ergene	1,892	65.2±6.1	5 (0.3±0.6)
Gala	1,788	61.7±5.5	145 (8.2±7.3)
Gonen	2,041	68.0±6.3	170 (8.3±5.1)
Halilbey	1,891	67.5±5.4	57 (3.1±6.1)
Hamzadere	1,697	60.6±6.3	70 (4.2±5.6)
Haziran	1,624	56.0±7.4	239 (14.7±8.8)
Ipsala	1,139	51.8±10.3	32 (2.8±3.5)
Kale	1,729	66.5±7.8	116 (6.6±7.9)
Karadeniz	1,944	64.8±6.1	85 (4.3±4.9)
Kargi	1,841	61.4±6.6	384 (21.2±12.2)
Kiral	1,828	67.7±7.1	73 (4.1±4.0)
Kirkpinar	1,797	59.9±7.2	7 (0.4±1.3)
Kizilirmak	1,247	59.4±9.4	58 (4.5±2.4)
Kuplu	1,984	66.1±7.0	128 (6.4±4.4)
Manyas Yildizi	1,600	64.0±8.4	2 (0.1±0.6)
Meric	1,893	63.1±7.5	10 (0.6±1.1)
Mevlutbey	1,851	66.1±5.4	101 (5.5±5.1)
Negis	1,871	62.4±6.5	157 (9.0±10.1)
Osmancik-97	1,337	44.6±6.7	468 (35.1±13.7)
Pasali	1,968	70.3±7.6	39 (1.9±1.8)
Sumnu	2,059	71.0±9.2	36 (1.7±2.0)
Surek M1711	1,385	63.0±11.5	232 (16.4±10.0)
Surek 95	1,777	59.2±7.1	851 (48.9±16.2)
Tosya Gunesi	1,774	61.2±5.8	60 (3.4±2.8)
Trakya	1,964	64.1±7.5	94 (4.7±4.1)
Tunca	2,041	70.4±8.3	226 (11.0±10.0)
Ulfet	1,556	67.7±4.6	194 (12.3±8.2)
Yatkin	1,440	68.6±6.4	166 (11.3±8.1)
Yavuz	1,495	68.0±8.5	8 (0.5±1.0)
Average			**9.9±6.4**
Baekilmi*	2,037	67.9±7.3	191 (9.3±5.1)
Nipponbare*	1,561	52.1±5.5	260 (16.8±7.0)
Baldo*	1,914	63.8±6.8	7 (0.4±1.3)

*Reference cultivar for Korean (Baekilmi), Japanese (Nipponbare) and Italian (Baldo).Reference cultivar for Korean (Baekilmi), Japanese (Nipponbare) and Italian (Baldo).

The constraints associated with the anther culture of Turkish rice varieties

However, anther culture of Turkish rice varieties presents certain challenges. One major constrant is harvesting samples at the appropriate developmental stage. Spikeles are typically preselected based on the distance between the interligule of the flag leaf and the second leaf, as well as the position of the anther tip within the spikelet (Afza et al. 2000). The optimal timing for anther culture of Korean varieties was suggested to be when the distance between the flag leaf and the

Table 5. Regeneration of green and albino plants in the anther culture of Turkish rice varieties

Cultivar	No. of anthers induced callus	No. of plant regenerated			
		Sum	Green (A, %)	Albino (B, %)	A/B
Altinyazi	64	25	16 (25.0)	9 (14.1)	1.8
Bafra Yildizi	278	116	39 (14.0)	77 (27.7)	0.5
Beser	116	35	32 (27.6)	3 (2.6)	11
Biga Incisi	232	63	14 (6.0)	49 (21.1)	0.3
Cakmak	309	54	31 (10.0)	23 (7.4)	1.3
Demir	201	111	20 (10.0)	91 (45.3)	0.2
Ece	528	54	29 (5.5)	25 (4.7)	1.2
Edirne	57	11	5 (8.8)	6 (10.5)	0.8
Efe	553	57	13 (2.4)	44 (8.0)	0.3
Efsane CL	201	27	16 (8.0)	11 (5.5)	1.5
Ergene	5	0	0 (0.0)	0 (0.0)	-
Gala	145	97	39 (26.9)	58 (40.0)	0.7
Gonen	170	53	22 (12.9)	31 (18.2)	0.7
Halilbey	57	1	1 (1.8)	-	ns
Hamzadere	70	7	2 (2.9)	5 (7.1)	0.4
Haziran	239	64	11 (4.6)	53 (22.2)	0.2
Ipsala	32	19	7 (21.9)	12 (37.5)	0.6
Kale	116	77	24 (20.7)	53 (45.7)	0.5
Karadeniz	85	19	6 (7.1)	13 (15.3)	0.5
Kargi	384	105	16 (4.2)	89 (23.2)	0.2
Kiral	73	2	1 (1.4)	1 (1.4)	1
Kirkpinar	7	3	1 (14.3)	2 (28.6)	0.5
Kizilirmak	58	25	1 (1.7)	24 (41.4)	0
Kuplu	128	43	10 (7.8)	33 (25.8)	0.3
Manyas Yildizi	2	0	-	-	-
Meric	10	5	1 (10.0)	4 (40.0)	0.3
Mevlutbey	101	69	7 (6.9)	62 (61.4)	0.1
Negis	157	40	15 (9.6)	25 (15.9)	0.6
Osmancik-97	468	58	5 (1.1)	53 (11.3)	0.1
Pasali	39	18	2 (5.1)	16 (41.0)	0.1
Sumnu	36	8	3 (8.3)	5 (13.9)	0.6
Surek M1711	232	80	17 (7.3)	63 (27.2)	0.3
Surek 95	851	1172	520 (61.1)	652 (76.6)	0.8
Tosya Gunesi	60	0	0 (0.0)	0 (0.0)	-
Trakya	94	16	11 (11.7)	5 (5.3)	2.2
Tunca	226	55	20 (8.8)	35 (15.5)	0.6
Ulfet	194	32	7 (3.6)	25 (12.9)	0.3
Yatkin	166	76	21 (12.7)	55 (33.1)	0.4
Yavuz	8	1	0 (0.0)	1 (12.5)	ns
Average (%)			**10**	**31.1**	**0.3**
Baekilmi*	191	29	8 (4.2)	21 (11.0)	0.4
Nipponbare*	260	119	89 (34.2)	30 (11.5)	3
Baldo*	7	3	1 (14.3)	2 (28.6)	0.5

*Reference cultivar for Korean (Baekilmi), Japanese (Nipponbare) and Italian (Baldo)

second leaf's interligule was around 5 cm (Sohn et al. 1985), whereas for some Turkish varieties, it was suggested to be when the spikelets were exposed from the leaf sheath enclosing the panicle at around 5 cm (Figure 5). Exposed spikelets can also increase the risk of bacterial or fungal infections during the cold treatment period. While surface sterilization of spikelets is a potential solution, careless sterilization could adversely affect anther response or lead to increased contamination. Alternatively, adding antibiotics to the medium has been suggested as a solution (Thanh and Neeti 2015). However, further research is needed to determine the appropriate type and optimal concentration of antibiotic, and its potential impact on callus induction or plant regeneration. Additionally, to expand the application of rice anther culture in haploid breeding programs in Turkey, further studies should focus on improving differentiation rates and reducing albino expression.

The second constraint is the donor plant for anther culture. In Turkish rice varieties, callus induction and plant regeneration rates were variety-specific. Green plant regeneration was primarily influenced by maternal effects with lesser influence of gametic additive effects (Yan et al. 1996). These rates are affected by multiple factors, including the donor plant's genotype, nutritional status and external climate conditions as well as *in vitro* factors such as medium composition, additives, hormone ballaance, and culture conditions (Mishra and Rao, 2016). In this study, the average callus induction and plant regeneration rate were were about 10%. Further optimization of hormone composition, carbon source, and culture conditions is necessary to improve culture efficiency. Reduced levels of NH_4^+ and NO_3^{2-} in the medium (Grimes et al. 1990) and the use of maltose as an alternative carbon source (Yi et al. 2003) have been reported to enhance callus induction in the anther culture of rice. The genetic ancestor of European rice, often used as a breeding resource for Turkish rice, was tropical *japonica* (Brigitte, 2012). Tropical *japonica* is advantageous over temperate Mediterranean *japonica* and *indica* in anther culture (Camilo et al. 2018).

The third is albinism. In Turkish rice anther culture, albino plants were regenerated at three times the rate of green plants, posing a significant challenge for haploid breeding. Albinism is influenced by various factors, including the genotype and physiological state of the donor plant (Bullok et al. 1982), the temperature and duration of pretreatment, and the composition of the culture medium (Talebi et al. 2007). The fundamental cause of albinism is degredation of nuclear and plastid DNA (Kumari et al. 2009). It can be mitigated by shortening the culture period and transferring calli to regeneration media ealier (Asaduzzaman et al. 2003). A previous study observed simultaneous application of starvation and cold stress for shorter durations increased microspore survival but also led to higher frequencies of albinism (Touraev et al. 2009).

CONCLUSION

To successfully integrate rice anther culture into the haploid breeding program in Turkey, several challenges need to be addressed, including bacterial contamination, improving the green plant regeneration rate, and reducing albino plant occurrence. This study found that the average regeneration rate of green plants in the anther culture of Turkish rice varieties was approximately 10%. Despite variations among the varieties, our study suggests that haploid breeding technology canbe implemented in Turkish rice breeding programs is feasible on a limited scale. In Turkey, rice breeding primarily employs the drill-seeding method for the selection of early generations. However, compared to transplantation, drill-seeding poses a higher risks of environmental variation and contamination from volunteer plants. Haploid breeding offers the advantage of obtaining homozygotes in a single generation, with high selection efficiency. Furthermore anther culture is expected to be an an effective tool for the early introduction of traits like the IMI-R trait into existing varieties.

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