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PRODUCTION OF HAPLOIDS IN ANCIENT, LOCAL AND MODERN WHEAT BY ANTHER CULTURE AND MAIZE POLLINATION

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Our study involved the first-ever evaluation of the performance of anther culture and wheat × maize hybridization techniques in producing haploids or doubled haploids as a result of spontaneous doubling of the chromosome number during androgenesis in plants from 30 wheat genotypes including ancient, local and modern types. The results indicated that the best induction rates of androgenic structures and haploid embryos for the hexaploid and tetraploid wheat genotypes were obtained with anther culture and wheat × maize hybridization, respectively. Whereas only one regenerated plant from 15 genotypes of tetraploid wheat was obtained, 13 plants were regenerated from 15 genotypes of hexaploid wheat. Moreover, haploid embryos obtained in wheat × maize hybridization 60 and 100% green plants regenerated in relation to the number of the cultured haploid embryos. Genotypes with high induction capacity to produce androgenic structure or haploid embryos did not have desired haploid plantlets regeneration capacity and vice-versa. However, with both methods, hexaploid wheat genotypes had a considerable ability to produce green plants. Doubled haploid plants were obtained from ancient and local wheat genotypes by both methods, but not from modern wheat. Those genotypes can be used as parents in future wheat breeding programs and new varieties may be obtained by selecting pure lines in wheat populations.

Keywords: androgenesis, embryo rescue, haploidization, interspecific crosses, tetraploid and hexaploid wheats

INTRODUCTION

Among thousands of *Triticum* species identified, the ones of commercial significance belong to the hexaploid (*Triticum aestivum* L.) or tetraploid (*Triticum durum* Desf.) wheat species resulting from natural hybridization of diploid ancestors. Those varieties have thrived due to breeding programs aimed at optimizing not only their yields but also technological features for industrial production of bread, pasta and many other products. However, in response, cultivation of those ancient and local wheat species has diminished because modern wheat varieties simply yield more (Mefleh et al., 2019).

Even so, today's consumers, farmers and food producers are regaining interest in ancient wheat varieties. Among them, einkorn, emmer, spelt, Polish and Khorasan wheat (Kamut) have all been increasingly reproduced due to their low need for fertilizer, high adaptability and genetic diversity. Various studies have also suggested that those wheat varieties possess health benefits and even potential value of reducing the incidence of celiac disease related to consumption of wheat-based foods (Bordoni et al., 2017; Boukid et al., 2018; Bienkowska et al., 2019). *Triticum turanicum* Jakubz., *Triticum polonicum* L. and *Triticum sphaerococcum* Perc. have become especially re-

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nowned for their disease resistance, use in production of functional foods with health-promoting properties and organic cultivation (Janković et al., 2020; Suchowilska et al., 2020; Szczepanek et al., 2020).

The same shift from traditional production systems to environmentally friendly ones has created new opportunities for local wheat varieties as well. Local wheat varieties are genotypes with population characteristics that have been developed by farmers using traditional methods and adapted to a certain region with the effect of natural selection. The variation in local wheat populations is extensive and they can be used as a source of genes in breeding programs focused on the protein ratio, coleoptile length, salt tolerance, earliness, resistance to biotic and abiotic stresses, and mineral, antioxidant and lutein content (Morgounov et al., 2016). In fact, integrating the ancient and local wheat varieties in breeding programs is essential for boosting yields and transferring certain characteristics to high yield modern wheats. Beyond that, to develop superior varieties from them, it is also essential to understand their capabilities in doubled haploid plant production.

Obtaining haploid plants from hybrids followed by chromosome doubling to develop 100% homozygous lines provides an important way for wheat breeders to accelerate the development of varieties. Doubled haploid populations are used in various activities including development of the cultivar and germplasm, transfer of traits from wild types, study of components of quantitative genetics, and whole genome mapping. Moreover, as Lantos and Pauk (2020) reported in their review, doubled haploid plant production techniques allow for rapid selection of recessive alleles. The most common haploid wheat breeding techniques are anther culture and chromosome elimination via wheat × maize hybridization. In comparison, however, maize mediated doubled haploid plant production technique is more efficient than anther culture, owing to the low level of genotype specificity required, the absence of albinism and relative ease of application (Patial et al., 2019).

By contrast, anther culture has been used to develop several cultivated wheat varieties, but its effectiveness suffers from the influence of the wheat genotype (Baenziger and DePauw, 2009). Nevertheless, genotypes that are not susceptible to callus induction and plantlet production in anther culture can be good parents for chromosome elimination with wheat × maize hybridization (Campbell et al., 2000). The technique is expensive, but the short time needed to develop a variety compensates for high cost (Patial et al., 2019).

In both techniques, the ability to produce haploid plant varies among species and within each species. In short, some genotypes of the same species demonstrate excellent responses, whereas others may be intractable. Reynolds (1997), Labbani et al. (2007), and Slama-Ayed et al. (2019) have all reported that durum wheat genotypes are less responsive to anther culture than hexaploid wheat genotypes. Meanwhile, Ltifi et al. (2019) has argued that the success in wheat × maize hybridization largely depends on the durum wheat genotype. The genotypic effect on haploid embryo production in the wheat × maize system also remains a controversial phenomenon in the literature. In our previous studies, we established that the genotype is pivotal in both anther culture and wheat × maize hybridization (Yorgancilar et al., 2015, 2016, 2017; Kutlu et al., 2019; Avci and Kutlu, 2020).

Before launching a crossbreeding program for the superior properties of the precious material used in studies, it is appropriate to determine their potential for haploid plant production. Therefore, we aimed to investigate the extent to which is beneficial to use both anther culture and wheat × maize hybridization to produce haploid plants of 30 wheat genotypes (15 tetraploids and 15 hexaploids) including ancient, local and modern varieties. In so doing, we sought to support the selection of suitable genotypes with high regenerative ability for future hybridization studies.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS OF DONOR PLANTS

Fifteen tetraploid and 15 hexaploid wheat genotypes with different agricultural properties were used as donor plants and information about them is presented in Table 1. Ancient and local wheats were provided from Gene Bank of U.S. Department of Agriculture. The modern wheat cultivars were provided from Transitional Zone Agricultural Research Institute in Eskisehir. Wheat plants were grown in 14 cm pots containing soil, peat and vermiculite (3:2:1) in a plant growing room adjusted to 22°C by day and 15°C at night,

TABLE 1. Wheat genotypes used in the study.

Tetraploid wheats				
No	**Plant ID**	**Plant name**	**Species**	**Plant induction**
1	PI 10391	CItr 2431	*Triticum turanicum*	Ancient
2	CItr 14139	CI 14139	*Triticum polonicum*	Ancient
3	PI 349058	Kara Gylchyh	*Triticum turgidum*	Ancient
4	PI 165103	Menceki	*Triticum durum*	Local
5	PI 165134	Akbasak	*Triticum durum*	Local
6	PI 165137	Kunduru	*Triticum durum*	Local
7	PI 165152	Sahman	*Triticum durum*	Local
8	PI 166815	Menceki	*Triticum durum*	Local
9	PI 166850	Uveyik	*Triticum durum*	Local
10	PI 178141	Kose	*Triticum durum*	Local
11	PI 341353	Sari Kilcik	*Triticum durum*	Local
12	PI 166728	Kadiroglu	*Triticum durum*	Local
13		Eminbey	*Triticum durum*	Modern
14		Kumbet	*Triticum durum*	Modern
15		Kiziltan	*Triticum durum*	Modern
Hexaploid wheats				
No	**Plant ID**	**Plant Name**	**Species**	**Plant Induction**
1	PI 41023	Termok	*Triticum compactum*	Ancient
2	PI 140191	6332	*Triticum macha*	Ancient
3	CItr 8610	52	*Triticum sphaerococcum*	Ancient
4	PI 24485	Kara Bugdai	*Triticum aestivum*	Local
5	PI 166276	Kose	*Triticum aestivum*	Local
6	PI 166303	Sunter	*Triticum aestivum*	Local
7	PI 166726	Kadiroglu	*Triticum aestivum*	Local
8	PI 166740	Saribasak	*Triticum aestivum*	Local
9	PI 166762	Kirik	*Triticum aestivum*	Local
10	PI 166790	Tir	*Triticum aestivum*	Local
11	PI 166869	Menceki	*Triticum aestivum*	Local
12	PI 173500	Asure	*Triticum aestivum*	Local
13		Reis	*Triticum aestivum*	Modern
14		Mufitbey	*Triticum aestivum*	Modern
15		Esperia	*Triticum aestivum*	Modern

60–70% humidity, 450 μmol m^{-2} s^{-1} photosynthetic flux density and 16/8 h lighting period, after being vernalized at 4°C for 5 weeks.

Four sugar maize varieties were used as pollen sources including Baron, Challenger, Earliking, and Merit for wheat × maize hybridization. Baron and Merit were provided from May Seed Company, Challenger from Seminis Seed Company, and Earliking from NutsnCones Seed Company. Maize plants were grown in 24 cm pots containing the

same mixture in a greenhouse at 28°C by day and 17°C at night, 800 µmol m^{-2} s^{-1} photosynthetic flux density and 14/10 h lighting period. Maize seeds were sown 4 times at 7 day intervals, in order to synchronize the wheat flowering times with pollen production of maize. Both wheat and maize plants were applied with a liquid fertilizer including macro and micro plant nutrients once in two weeks.

WHEAT × MAIZE HYBRIDIZATION TECHNIQUE

Wheat spikes were emasculated once their spikes had emerged slightly from the flag leaves, and were covered with isolation envelopes (Fig. 1a). The maize pollens were freshly collected in a mixture after 1–3 days after emasculation (Fig. 1b), and used to pollinate the spikes of each wheat genotype (Fig. 1c). Afterwards, pollinated spikes were sprayed with 2,4-D solution (213.05 mg/l, pH = 10.36) after 24–48 h following hybridization to support healthy embryo formation as described by Niu et al. (2014) (Fig. 1d).

The formation of pseudo-seeds was observed in the wheat spikes 16–19 days after applications (Fig. 1e). The picked pseudo-seeds were sterilized in 70% alcohol for 1 min (Fig. 1f), placed in 20% bleach (Domestos, 4.26% sodium hypochlorite) for 15 min, and then rinsed with sterile distilled water at least 3 times. The haploid embryos were carefully removed from the pseudo-seeds under stereo microscopy (Fig. 1g), cultured on MS medium (Murashige and Skoog, 1962) containing 5% sugar and 0.7% agar, and kept in the dark at 22±0.5°C for 1–2 weeks (Figs. 1h,i). Germinated embryos were transferred in ½ MS media containing 3% sugar and 0.7% agar and kept for 2 weeks in a 16/8 light cycle at 22±0.5°C (Fig. 1j).

ANTHER CULTURE TECHNIQUE

Donor tillers from each genotype collected when the microspores were in early uninucleate stage, were first exposed to a pre-cold application at 4°C for 13–14 days in Erlenmeyer flasks containing tap water and covered with PVC bags. Afterwards, the spikes were removed from the stem and leaves, and sterilized with 2% sodium hypochlorite with 1–2 drops of Tween-20. After sterilization, the anthers were picked up from the spikes with sterile forceps in a sterile cabin (Fig. 2a) and 100 anthers in four replicates were planted in the previously prepared MN6 induction media (Ouyang, 1986) with components listed in Table 2

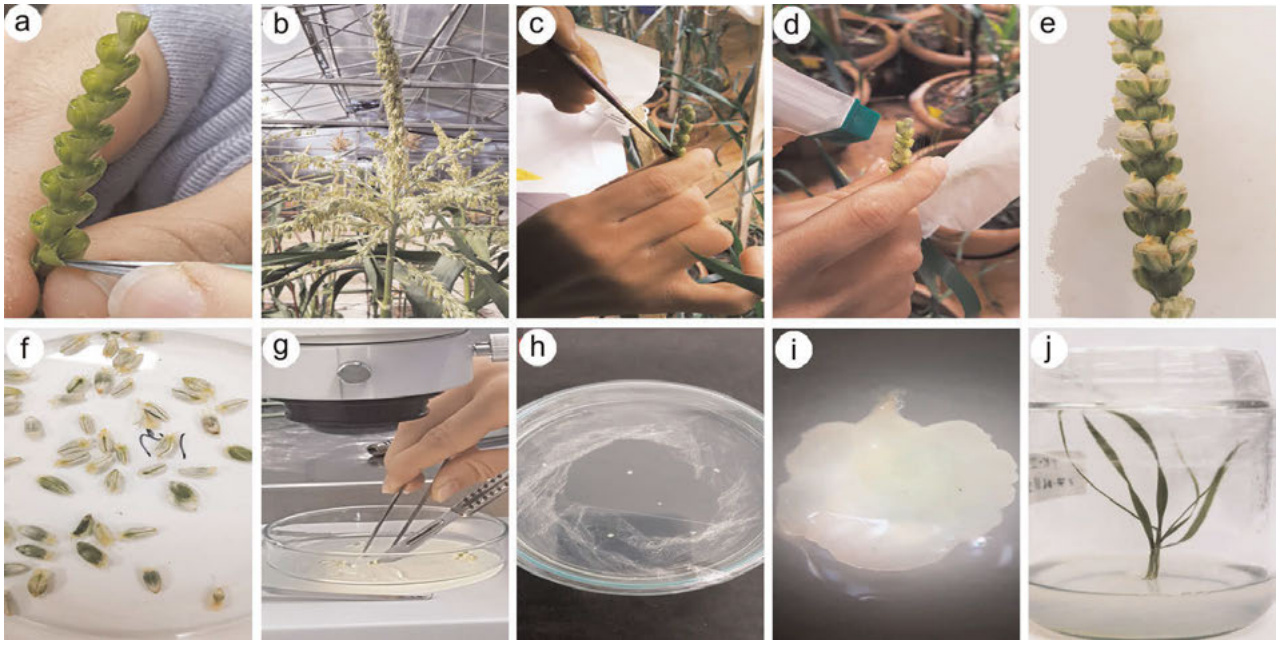

Fig. 1. Haploid plant production process using wheat x maize hybridization technique. (**a**) emasculation of wheat spikes (**b**) collection of pollen from maize plants (**c**) pollination of florets of wheat (**d**) spraying hormone on wheat spikes (**e**) pseudo-seed formation on spike (**f**) harvested pseudo-seeds (**g**) rescue of embryos (**h**) rescued embryos on culture media (**i**) haploid embryo (**j**) haploid plants developed from embryo.

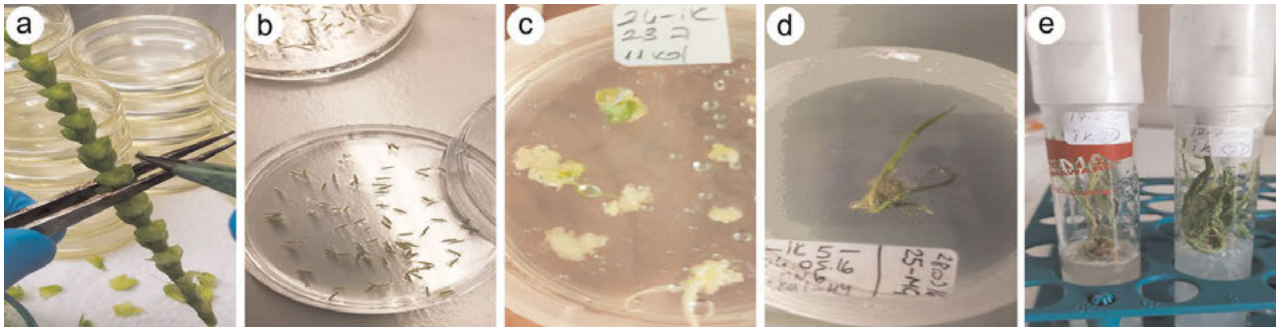

Fig. 2. Haploid plant production process using anther culture technique. (**a**) picking up anthers (**b**) planted anthers on culture media (**c**) androgenic structures development in Kirik bread wheat genotypes (**d**) plantlet development in Kirik bread wheat genotypes (**e**) plantlets transferred into test tubes.

(Fig. 2b). The cultures were kept in a dark incubator at 28°C until induction of androgenic structures, which, once obtained, were transferred to 190-II-Cu nutrient media (Zhuang and Jia, 1980; Table 2) for green plant regeneration at 25°C and in a 16/8 h lighting period for 30 days (Fig. 2c). Developing shoots from androgenic structures (Fig. 2d) were transferred to test tubes containing 190-II Cu rooting medium. Plantlets showing improvement were maintained in a growth chamber at 8°C for 16 h of light and at 4°C for 8 h of dark for 6 weeks (Fig. 2e).

TABLE 2. Components of induction (MN6) and regeneration (190-II Cu) media.

MN6		**190-II Cu**	
Component	**Amount (mg/l)**	**Component**	**Amount (mg/l)**
KNO_3	1150	KNO_3	100
$(NH_4)_2SO_4$ x $2H_2O$	100	$(NH_4)_2SO_4$	200
$Ca(NO_3)_2$ x $4H_2O$	100	$Ca(NO_3)_2$x $4H_2O$	100
$(NH_4)_2$ SO_4	80	KH_2PO_4	300
$MgSO_4$ x $7H_2O$	125	$MgSO_4$ x $7H_2O$	200
KH_2PO_4	200	KCl	40
KCl	35	Fe-Na-EDTA	20
2,4-D	1.5	$MnSO_4$ x $4H_2O$	8
Kinetin	0.5	$ZnSO_4$ x $7H_2O$	3
Ficoll	100.000	H_3BO_3	3
		Kl	0.5
		Glicine	2
Fe-Na-EDTA	5	Thiamine-HCl	1
Thiamine-HCl	1	Pyridoxine-HCl	0.5
Maltose	100	Nicotinic acid	0.5
		Myo-inositol	100
		Sucrose	0.5
		NAA	0.5
		Kinetin	0.5
		$CuSO_4$ x $5H_2O$	5.7

*The pH was adjusted as 5.8 in both media.

CHROMOSOME OBSERVATIONS AND COLCHICINE TREATMENT IN BOTH TECHNIQUES

When haploid seedlings grew to 5–6 cm, they were transferred to pots containing peat and vermiculite (3:1) for two weeks at 16°C with 16/8 h in light/dark period for acclimatization (Fig. 3a). When the seedlings had 2–3 tillers, their ploidy levels were checked by counting the chromosome number, as described by Maluszynska (2003) (Fig. 3b,c). While spontaneous doubled haploid plants derived from microspores, they were transferred directly to the growth chamber at 18±0.5°C with 75% humidity. The plants of haploid chromosome levels were doubled by applying colchicine solution: colchicine (0.45 g/l) + DMSO (20 ml/l) + GA_3(100 mg/l) + Tween 80 (0.3 ml/l), pH = 5.5, at 20–22°C, dark for 8 h, according to Niu et al. (2014) (Figs. 3d,e).

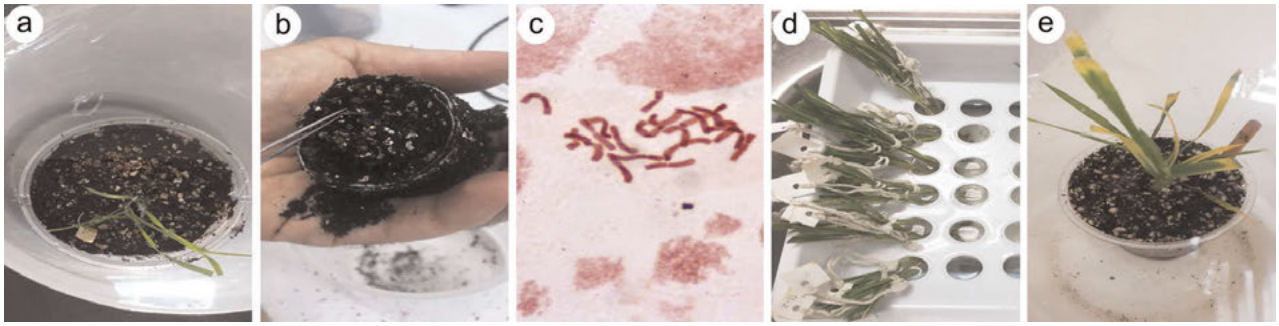

Fig. 3. Chromosome doubling process in both technique. (**a**) acclimation of haploid plants (**b**) selection of root tips for chromosome counting (**c**) haploid bread wheat chromosomes (**d**) colchicine treatment (**e**) transplanted plant after chromosome doubling.

DATA COLLECTION AND STATISTICAL ANALYSES

Data concerning the pollinated florets, developed pseudo-seeds, haploid embryos, produced androgenic structures and regenerated plantlets were collected, and statistical comparisons were performed with a chi-square test. The frequencies of the collected data were calculated using the following formulas:

Pseudo-seeds frequency = number of pseudo-seeds / pollinated florets × 100

Haploid embryos frequency = number of haploid embryos / pseudo-seeds × 100

Plantlets frequency = number of plantlets / haploid embryos × 100

Androgenic structures frequency = number of androgenic structures / anthers incubated × 100

Spontaneous doubled haploid or haploid plants frequency = number of plants / androgenic structures × 100

RESULTS AND DISCUSSION

Determination of the potential of haploid plant regeneration of wheat genotypes is critical before commencing wheat breeding programs. In our study, the effectiveness of two methods of haploidization in the haploid regeneration of 30 wheat genotypes was compared. In the wheat × maize hybridization method, 5,117 florets of wheat pollinated with maize pollens produced 26.2% pseudo-seeds, and the greatest frequency of pseudo-seeds was obtained in *T. sphaerococcum* (Table 3). However, the greatest number of pseudo-seeds, 123, belonged to *Triticum compactum* L., followed by *Triticum turgidum* L. cv. Kara Gylchyh with 112 pseudo-seeds (Fig. 4). Whereas all

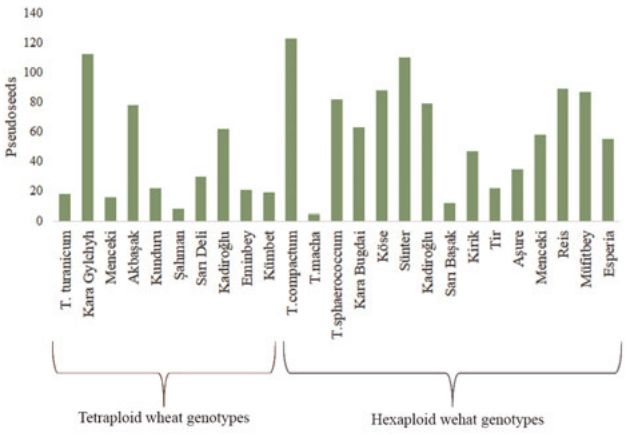

Fig. 4. Number of pseudo-seeds (χ^2: 151.52**)

TABLE 3. Frequencies of pseudo-seeds, embryos, androgenic structures and plantlets.

	Genotype	Number of pollinated flowers	Frequency of pseudo seeds	Frequency of haploid embryos	Frequency of plantlets	Number of isolated anthers	Frequency of androgenic structures	Frequency of haploid plantlets	Frequency of spontaneous doubled haploid plantlets
Hexaploid wheats	*T. turanicum*	154	11.7	–	–	400	1.7	–	14.3
	T.polanicum	158	–	–	–	400	–	–	–
	Kara Gylchyh	204	54.9	4.5	60.0	400	–	–	–
	Menceki	100	16.0	–	–	400	–	–	–
	Akbasak	226	34.5	–	–	400	–	–	–
	Kunduru	196	11.2	4.5	100.0	400	–	–	–
	Sahman	200	4.0	–	–	400	–	–	–
	Sari Deli	206	14.6	3.3	–	400	–	–	–
	Uveyik	176	–	–	–	400	–	–	–
	Kose	136	–	–	–	400	–	–	–
	Sari Kilcik	180	–	–	–	400	–	–	–
	Kadiroglu	200	31.0	4.8	–	400	–	–	–
	Eminbey	200	10.5	–	–	400	–	–	–
	Kumbet	170	11.2	–	–	400	–	–	–
	Kiziltan	170	–	–	–	400	0.2	–	–
	T.compactum	196	62.8	3.2	25.0	400	–	–	–
	T.macha	198	2.5	–	–	400	–	–	–
	T.sphaerococcum	108	75.9	–	–	400	–	–	–
Tetraploid wheats	Kara Bugdai	180	35.0	9.5	33.3	400	–	–	–
	Kose	186	47.3	20.4	16.7	400	0.2	–	–
	Sunter	206	53.4	7.3	–	400	3.0	16.7	–
	Kadiroglu	170	46.5	–	–	400	–	–	–
	Sari Basak	162	7.4	–	–	400	0.2	–	–
	Kirik	160	29.4	2.1	–	400	9.5	13.2	13.2
	Tir	152	14.5	9.1	–	400	11.5	–	–
	Asure	160	21.9	2.9	–	400	3.2	7.7	–
	Menceki	162	35.8	5.2	–	400	–	–	–
	Reis	128	69.5	7.9	–	400	–	–	–
	Mufitbey	152	57.2	3.4	–	400	1.0	–	–
	Esperia	121	45.4	3.6	–	400	2.5	–	–
	Total (number)	**5117**	**1341**	**65**	**10**	**12000**	**133**	**8**	**6**

hexaploid wheat genotypes produced pseudo-seeds, the tetraploid wheat genotypes including *T. polonicum*, Uveyik, Kose, Sarı Kilcik and Kızıltan did not. The genotype that produced the fewest pseudo-seeds was *Triticum macha*.

Although, pseudo-seed production can be considered an indicator of success of wheat × maize hybridization, to contain the healthy haploid embryo of those seeds is an essential criterion indicating the genotype's suitability for the technique. Most embryos abort during the initial stage of development due to the poor viability of zygotes, which reduces the number of embryos converted into plantlets (Chaudhary et al., 2015; Slama-Ayed et al., 2019). Similarly, the success of the first step of anther culture can be measured by the number of androgenic structures acquired. In our study, 65 (4.8 %) embryos were rescued from 1,341 pseudo-seeds, and 133 androgenic structures (1.1%) were obtained from 12,000 incubated anthers (Table 3). Among the tetraploid wheats, *T. turanicum* from ancient wheats did not produce haploid embryos *via* wheat × maize hybridization, but it produced seven (1.7%) androgenic structures via anther culture (Fig. 5). Most haploid embryo production (5) occurred in the *T. turgidum* cv. Kara Gylchyh genotype. While only Kunduru, Sari Deli and Kadiroglu produced haploid embryos in local tetraploid wheats, haploid embryos could not be obtained from any modern varieties. Among the modern tetraploid wheat genotypes, Kızıltan responded to the anther culture by including only one androgenic structure. Low responses to both interspecific crossbreeding and anther culture have also been reported by Sharma et al. (2019), Slama-Ayed et al. (2019) and Kapoor et al. (2020) for durum wheats. The greatest haploid embryo production among the hexaploid wheats occurred in local wheat genotypes Kose with 18 haploid embryos and Sunter with 8 haploid embryos. Kirik and Tir local wheats with 38 and 46 androgenic structures, respectively, were the most prominent varieties (Fig. 5). Kose, Sunter, Kirik, Tir, Asure, Mufitbey, and Esperia responded to both methods with haploid embryo and androgenic structures production. The marked anther response of the Mufitbey and Esperia modern bread wheat varieties was also obtained in our earlier research (Yorgancilar et al., 2017).

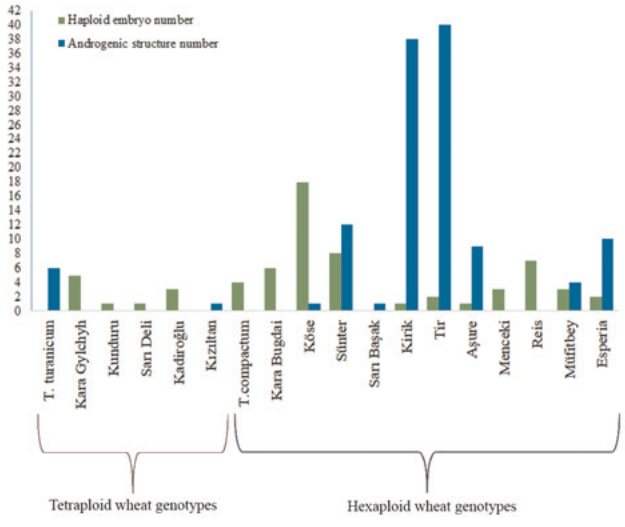

Fig. 5. Number of haploid embryo and androgenic structures (χ^2 {haploid embryo}: 52.06**; χ^2 {androgenic structures}: 178.61**)

Although the induction of androgenic structures and haploid embryos indicates haploid production, plant regeneration is the key step in both methods. Our study revealed that genotypes which produce androgenic structures or haploid embryos lack a good capacity to regenerate haploids in both methods, and vice-versa. For example, Tir, despite its relatively good induction of androgenic structures via anther culture, demonstrated no capacity to regenerate haploids. Kara Gylchyh, meanwhile, was able to produce haploid plants well in wheat × maize hybridization. *T. turgidum* cv. Kara Gylchyh, Kunduru, *T. compactum*, Kara Bugdai, and Kose haploids regenerated well with wheat × maize hybridization, while *T. turanicum*, Sunter, Kirik, and Asure responded better to anther culture (Fig. 6). Jauhar (2003) and Cistué et al. (2009) characterized durum wheat, a species recalcitrant to androgenesis including anther and microspore cultures, as showing a low regenerative capacity and a high frequency of albino plants. In our study, albino plants were not produced and only one green plant from *T. turanicum* was obtained.

Although anther culture has not demonstrated success in producing haploids of durum wheat (Dogramaci-Altuntepe et al., 2001), wheat × maize hybridization has proven efficiency in producing haploid durum wheats (Almouslem et al., 1998). However, hexaploid bread wheat genotypes have been more crossable with maize than durum wheat genotypes. The successful crossability of bread wheat could be attributed to the presence of the D genome. Kapoor et al. (2020) evaluated haploid plant production by crossing maize and

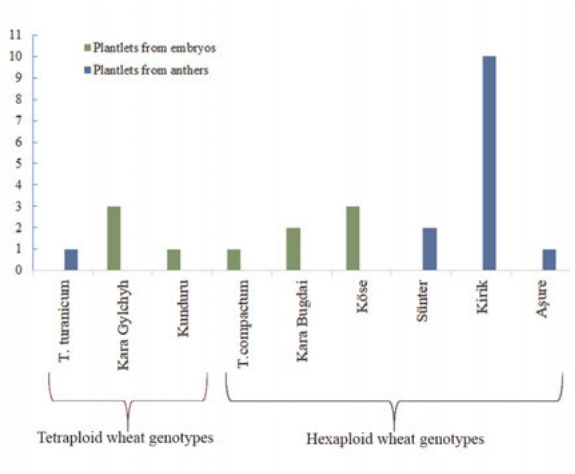

Fig. 6. Number of plantlets (χ^2 {plantlet from embryo}: 6.36**; χ^2 {plantlet from androgenic structures}: 8.05**)

Imperata cylindrica with tetraploid wheat, hexaploid wheat and hexaploid triticale. Like in our study, they found that the production of pseudoseeds and haploid embryos in hexaploid wheat was higher than in tetraploid wheat, and that plant regeneration was close to each other. Therefore, they proposed that the D genome in hexaploid wheats triggered the chromosome elimination of the paternal parent.

The D genome plays an important role in the wheat's response to anther culture as well as wheat × maize hybridization. Lazaridou et al. (2016) reported that some chromosomes belonging to the D genome are effective in the androgenic ability of wheat. They also observed that the green plant production potential in wheat may depend on possible interactions between the A, B and D genomes, which may consequently affect the androgenic response in bread wheat.

In our study, although some genotypes responded to both methods with the induction of haploid embryos and androgenic structures, there was no concurrence in the creation of green plants (Fig. 5). Those results confirm that the two methods are alternatives to each other, as our previous study showed (Avci and Kutlu, 2020). Despite the difficulty in obtaining haploid embryos, the high frequency of green plants makes the wheat × maize hybridization method more attractive than anther culture. The method's success in durum wheat is also notable. Dogramaci-Altuntepe and Jauhar (2001) reported that wheat × maize hybridization achieves a greater success when the 5B chromosome in durum wheat is substituted with the 5D chromosome in bread wheat. An additional B genome chromosome may have an impact analogous to the D genome chromosome. The absence of the D genome in durum wheat could be compensated by additional doses of the B genome (Dogramaci-Altuntepe and Jauhar, 2001). Thus, it may be possible to increase the success of haploid methods by using the Kara Gylchyh genotype in *T. durum* × *T. durum* hybridization or with pentaploid wheats obtained by *T. aestivum* × *T. durum* hybridization.

CONCLUSION

Our results show that both anther culture and wheat × maize hybridization between taxonomically diverse wheat genotypes can be successful. Most plants were obtained from the local bread wheats Kirik and Kose and the ancient durum wheat Kara Gylchyh. Those genotypes given their high nutritional quality, tolerance to stress and rich genetic variability are indispensable varieties both for breeding programs and for farmers in Turkey. In addition to their superior features, their outstanding capacity to regenerate haploids and their accessibility from gene banks may make the genotypes important for breeding programs around the world. Along with including those genotypes in crossbreeding programs, both haploidy methods can facilitate the acquisition of pure lines from ancient and local wheat genotypes. However, if plant production is limited, then such studies would be of limited interest. It is also clear that regenerating plants using conventional anther culture and wheat × maize hybridization is rather difficult. Therefore, the genetic mechanism behind plant regeneration should be investigated at the molecular level and the effect of the D genome investigated in greater depth, ideally by replicating the methods with pentaploid lines to be obtained from the hybridization of durum and bread wheats.

AUTHORS' CONTRIBUTIONS

All contributions of authors are equal and they have read and confirmed the final version of the manuscript for publication. The authors have declared that there is no conflict of interest.

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