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A Study on Usability of National Registered Durum Wheat Cultivars for Synthetic Bread Wheat Production

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ABSTRACT

The present study was conducted in Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary, from 7th September 2015 to 31st July 2016, and aimed to evaluate the crossabilities of 6 *Triticum durum* (Ç-1252, Kızıltan, Altıntaş, Dumlupınar Yelken and Kunduru) with 6 *Aegilops tauschii* (MVGB605, MVGB1323, MVGB589, CIMMYT224, CIMMYT372 and CIMMYT458). *Triticum durum* and *Aegilops tauschii* parents had been successfully grown, and the desired number of spikes had been emasculated and pollinated (total 171 spikes). Total 34 combinations were made and 164 embryos were obtained from 19 combinations among them. There were no embryos in 15 combinations. Number of maximum embryo rescue was obtained from Yelken/MVGB589 combination (24 embryos). It was followed by Kızıltan/MVGB589, Ç-1252/MVGB589 and Ç-1252/2T combinations (16, 13 and 11 embryos, respectively). 70 germinated plants were obtained from 164 embryos in B-5 medium (in petri dishes). 94 embryos were not germinated. A total of 29 germinated embryos died in two B-5 medium (petri dish and tube). Some older and younger tillers died after colchicine treatment. However, we had new shooting tillers. According to results, the best females were Yelken and Ç-1252 among *Triticum durum*s, while the best male MVGB589, CIMMYT224 and MVGB1323 among *Aegilops tauschii*s, respectively.

Keywords: hybrid, synthetic, wheat, *Ae. tauschii,* crossability

Introduction

Wheat has an important place in Turkey's national economy, and new varieties resistant to both biotic and abiotic stresses will play an important role in ensuring that wheat preserves its current status and importance (Shah *et al*., 1987; Cox *et al*., 1994; Mujeep-Kazi and Hettel, 1995; Mujeep-Kazi *et al*., 2008). In this context, it is important to consider the varieties and benefits which synthetic wheat will provide (Thompson and Zwart, 2008). Many countries all over the world have already begun to use synthetic wheats in production and breeding programs, and interest in synthetic wheats is gradually growing (Mujeep-Kazi *et al*., 2008).

For Turkey, the utilization of synthetic varieties in hybridization programs is of considerable importance. After being first hybridized, it may take up to 18 years for a new registered variety to be used in the production stages in farmers' fields. For this reason, it is important to use synthetic bread wheat varieties for developing new varieties for Turkey that have wide adaptability. Durum wheat, which is used as the source for synthetic bread wheat varieties, already represents a variety with significant adaptability. In this context, the current project plans to produce synthetic bread wheat varieties by utilizing local durum wheat genetic stocks.

Synthetic bread wheats obtained through interspecies have several disadvantages compared to cultivated bread wheats. However, extensive hybridization studies on culture wheats may reduce these disadvantages, or change them into advantages (Villareal *et al*., 1994; Lage *et al*., 2004; Mujeeb–Kazi *et al*., 2008). Compared to culture wheat, synthetic wheats are more resistant and/or hardy against biotic and abiotic environmental conditions. For example, Mujeep-Kazi and Hettel (1995) previously tested the salt resistance as well as the *Helminthosporium sativum, Fusarium graminearum,* and *Tilletia indica* resistance of various synthetic varieties obtained through the hybridization of durum wheats and *T. tauschii.* Based on the obtained results, they determined that the synthetic varieties were all comparatively more resistant to salt and these diseases than their parents. Synthetic hexaploids have 2n=3x=21 chromosomes. After chromosome doubling is performed with the chemical substance colchicine, the seeds generally have 42 chromosomes. However, hypoploids with 41 chromosomes and hyperploids with 43 chromosomes may also be encountered among the obtained synthetic varieties (Mujeep-Kazi and Hettel, 1995). This generally stems from differences in chromosome folding periods following colchicine application (Sears, 1941; Kihara, 1924; Sears, 1944; McFadden and Sears, 1946; Sears, 1956).

The most difficult stage of synthetic bread wheat production is the procedure used for embryo regeneration and chromosome doubling. For this reason, this project is necessary to observe the latest development in embryo culture and chromosome doubling methods at the Agricultural Institute of the Centre for Agricultural Research of the Hungarian Academy of Sciences, and for further increasing our knowledge on these procedures. Using materials from Turkey, the project will enrich our knowledge regarding synthetic wheat production based on the latest international developments and advances in this field. Owing to this project, we will be able to add information regarding the most recent advances in double haploid wheat production in Hungary to the current body of knowledge, and also initiate synthetic wheat production studies that will serve to broaden the genetic basis available in Turkey for breeding studies-which is particularly important at this time when the effects of global warming are being increasingly felt in Turkey.

Materials and Methods

1. Experimental parental materials and Planting

This study was started on 7th September in 2015. The experiments were primarily performed by using six durum wheat varieties (*Triticum durum*) originating from Turkey. These varieties were the Kunduru-1149, Altıntaş-95, Yelken-2000, Kızıltan-91, Ç-1252, and Dumlupınar (Table 1). Durum wheats were sown 8 seeds per variety in jiffy pod. All of durum wheats were sown at 7 different times with seven-day intervals. 8 *Aegilops tauschii*accessions were sown in jiffy pod as father (all of tauschii in 4th and 3 tauschii were replicated in 5th week) (Table 2; Figure 1). It would be ensured that the heading date of durum wheat varieties and the flowering date of *Aegilops tauschii* materials overlap.

2. Emasculation and pollination

3 *Aegilops tauschii* materials determined by the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and 1 *Aegilops tauschii* determined by the gene bank of the Centre for Agricultural Research of the Hungarian Academy of Sciences (MVGB) were used in crossing. Because 5 and 8 *Aegilops tauschii* accessions from CIMMYT have not had enough germinations. We did not use them in the crossing. We want to produce seeds from those *Aegilops tauschii* genotypes. 2 and 3 *Aegilops tauschii* accessions from the MVGB being too late for heading compared to durum wheat.

The heading date was 50% visible of first spike in leaf sheath and the flowering date was visible of the first anthers in spike. There were big differences among durum wheats and among *Aegilops tauschii* materials about heading and flowering. Durum wheats were used as female plants and *Aegilops tauschii* materials were used as male plants (William, *et al*., 1993; Mujeep–Kazi and Hettel, 1995). The crossing with pollen from the male plant was performed using the “twirl method” (Quisenberry, 1967).

2.4-D treatment is important for hybrid seed development (Koba *et al*., 1991). 2.4-D solution was prepared (0.05 g + 2 ml Ethanol + 100 ml bidistilled water) and had been successfully applied by injection into middle of upper internode of spike as soon as after pollination. After 2.4-D solution application, injection hole was covered with vaseline.

In a total 171 crosses were made (Table 3). In all of crosses, 6 durum wheat varieties were used as female and 6 *Aegilops tauschii* were used as male except 5T and 8T. 2D/4T and 4D/4T combinations were not made because of insufficient pollen. 5D/3T has had number of maximum cross (15) compared to other combinations.

3. Sterilization of seeds, embryo rescue, plant regeneration and vernalization

Embryo rescue studies have just been started at the end of February. The spikes and seeds were sterilized by following procedure;

The spikes were put in a bottle. A few drop of dishwashing liquid was added on the spikes. The bottle was filled up with water and shaken well, until the water starts to foam. The spikes were rinsed well with water (=3x).

%70 ethanol was added to the spikes and shaken for 10 min.

They were rinsed with sterile water (=4x).

In sterile box/laminar box:

The spikes in 5% Hypo (sodium hypochlorite) solution for 5 min were sterilized.

They were rinsed with sterile water (=3x).

0.1% $HgCl_2$ solution to the spikes was added, 3 min.

They were rinsed with sterile water (=4x).

Spikes of combination were harvested for spike and grain sterilization between 20 and 24 days after every crossing, (Figure 2). In the first term report, spike and grain sterilization were explained. Embryo rescue operations were started in 16 February 2016 and continued until to 29 May 2016. Embryos from all of hybrid seeds were rescued under microscope in sterilization-cabin (Sharma, 1999). B5 Agar Medium was prepared for regeneration of hybrid embryos and its growing.

Not only there was no endosperm or endosperm abnormality in hybrid grains (Kinoshita, 2007) but also there were no embryos in the majority of hybrid grains (Fujii and Toriyama, 2008) in spite of normal grain appearance (Figure 3). Clark and Wall (1996) reported that genomic relationship of two species was important for crossing. Therefore, crossabilities of species can have some barriers and difficulties. In interspecific hybridization, sometimes there are no hybrid grains or hybrid grains are smaller compared to normal grains (Stebbins, 1958; Linskens, 1972) (Figure 3). Mature seeds cannot be obtained from ovary culture or hybrid embryo because of abortion caused by the mismatch between embryo and endosperm development. Ovule and embryo culture methods are suitable (Bajaj *et al*., 1986). Embryo rescue operation is difficult because of smaller embryos. For this reason, 2.4-D hormone application is recommended for interspecific hybridization because of larger grain and embryo formation (Polgari *et al*., 2014) (Figure 3). Larger grains were created by 2.4-D hormone application.

Rescued embryos were put in B-5 medium in petrie dish (Gamborg *et al*., 1968). Maximum 5 embryos were put in every petrie dish. Petrie dishes were kept under dark and 20^0C condition for embryo germination during 10-13 days. After that, germinated embryos were put under light (14-16 hours per day) for the formation of chlorophyll to start the photosynthesis activity during 2 weeks (Figure 4A).

Haploid plants with sufficient root and shoot length were transferred to tube because of the limited space in petrie dishes (Figure 4B). One haploid plant was only planted in one tube. Tubes were kept under controlled condition (24^0C and 14-16 hours) during 1-3 weeks depending on root growth (Figure 4C). Haploid plants in tube were exposed to vernalization during 6 weeks (at $+2^0$C, and under 12 hours light and 12 hours dark) (Figure 4D). We had contaminations in some petrie dishes and tubes (Figure 5A and 5B). 12 haploid plants died due to contamination. Contaminated plants were 1D/3T (2 plants in 1 petrie dish), 2D/3T (4 plants in 1 petrie dish), 3D/7T (5 plant in 1 petrie dish) and 5D/3T (1 plant in 1 tube) combinations.

4. Transfer to soil

After vernalization, tubes (with plants) were taken to adaptation room (and under 14 hours light and 10 hours dark at 24^0C) during 1 week. After adaptation, plants were removed from tubes and the B-5 medium was washed off from roots thoroughly under tap water, and were transferred to pots (Figure 6A and B)). Planted pots were watered and covered by nylon to prevent moisture loss (Figure 6C). Nylons were removed from every pots after 1 week. Climate conditions per day of phytotron were illuminated during 12 hours at 15^0C and 65% humidity and darkened during 12 hours at 10^0C and 70% humidity. When younger plants are transferred from B-5 medium to soil, they are susceptible to lack of nutrient in the soil. Because of this reason, pots in phytotron were fertilized 2 times per week (on Monday and Thursday) with nutrient special solutions.

5. Colchicine treatment

In colchicine treatment, the following steps were performed;

The haploid plants one by one with sticker and pencil were labeled (Figure 7A),

The whole plants (with roots) were removed from pot,

Soil was washed off from roots thoroughly and bottom of The end of bottom of roots was cut 4-5 cm long (Figure 7B-C),

The plants were put into water (into a beaker) and stored them on 15°C until colchicine treatment,

0,04% colchicine ($C_{22}H_{25}NO_6$) solution was prepared:

To prepare 1000 ml solution, dissolve 0,4 g colchicine (Sigma) in a few drop of dimethyl-sulfoxide (Sigma-Aldrich), mix well and make volume up to 1000 ml (sterile water (MilliQ) or bidistilled water). It was tired at moderate speed.

The plants were put into the colchicine solution from 4.00 p.m. to 7.30 a.m. on 15°C (Figure 8A),

After colchicine treatment, the roots were washed thoroughly (minimum 2 hours) under flowing tap water, not too cold water (Figure 8B),

Plants were again planted into soil and leaves were cut 10 cm from upper level (Figure 9A and B),

The colchicine treated plants were put in phytotron under first condition (Figure 9C).

After colchicine treatment, some deformation and drying were observed on leaves and leaf sheaths of older and younger tillers (Figure 10A and B). Some older and younger tillers were also died by colchicine effect. These were an expected situation because colchicine was normally a very strong poison. New tillers shooted after colchicine treatment (Figure 11).

6. Calculation

Embryo Development Ability, Embryo Germination Ability, Vegetative Growth Ability in Tube and Soil Adaptation Ability for studied traits were calculated using the following formulas (modified from Yuan *et al*., 2016);

Embryo Development Ability
(EDA) = (RE/TF)*100
Embryo Germination Ability
(EGA) = (GE/RE)*100
Vegetative Growth Ability in Tube
(VGA) = (TP/RE)*100
Soil Adaptation Ability
(SAA) = (PS/PT)*100
Where;
TF = *Total Flowers*
RE = *Rescued Embryos*
GE = *Germinated Embryos in Petri-dish Medium*
PT = *Plants in Tube*
PS = *Plants in Soil*

Results

In this study, 171 spikes were pollinated in 34 combinations (a total of 5049 flowers) (Table 3). 164 rescued embryos (RE) were obtained from these crosses (Table 4). 5D female *Triticum durum* genotype had 48 embryos with 29.3% ratio among 164 RE. Because it had higher number of RE compared to other *Triticum durum* wheats (15). 6D female genotype had lowest RE value (9) with 5.5% ratio. According to germinated embryos in petri-dish (GE), GE ratios of 6D, 4D and 3D female genotypes were decreased (1.4%, 7.1% and 8.6%, respectively) compared to 5D, 2D and 1D (40%, 21.4% and 21.4%, respectively). These results were nearly same values of their offsprings in tube (PT). 5D and 1D genotypes among all female durum wheats had the best ratios of plants in soil (PS) trait (46.3% and 26.8, respectively) according to RE ratio (29.3% and 18.3%, respectively). 3T genotype was used maximum number of crosses (67 with 40.9% ratio) as male among all of *Aegilops tauschii*s. Others were nearly same each other's. Maximum GE ratio was observed in 3T male genotype (61.4), while minimum GE ratio was observed in 6T and 1T male genotypes (4.3% and 5.7%, respectively). PT ratios of *Aegilops tauscii*s did not nearly changed compared to GE. 3T, 4T and 2T genotypes among all male *Aegilops tauscii*s had the best ratios of PS trait (53.7%, 14.6 and 12.2, respectively) according to RE ratio (40.9%, 11% and 11.6%, respectively).

3.2% embryos were only obtained from 5049 pollinated flowers. Embryo development ability (EDA) of 5D/6T combination was the highest ratio (9.2%), but all of them were not interestingly germinated (Table 5). Maximum pollinated spikes were in 5D/3T combination (15 spikes) and, as expected, maximum number of embryos was obtained from this combination (24 embryos) (Table 6). 5D/3T combination had not only the highest EDA value (6.3%) but also the highest embryo germination ability (EGA) and vegetative growth ability in tube (VGA) (79.2% and 79.2%, respectively). 2D/3T combination had good values for EDA and EGA (5.3% and 56.3%, respectively). But its value was decreased in VGA (18.8%) and also it had zero (0%) in soil adaptation ability (SAA) value. The results of VGA values for each combination at the stage of colchicine treatment were more clear and important. After B-5 medium conditions, the best combinations about adaptation to soil were 1D/2T, 1D/3T, 1D/6T, 2D/1T 3D/T4 and 6D/4T (100%).

According to results, the best females were 5D and 1D among *Triticum durum*s, while the best female 3T, 4T and 2T among *Aegilops tauschii*s, respectively.

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Table 1. Code, species, accession and origin of experimental materials

Code	Species	Accession	Origin
1D	*T. durum*	Ç-1252	TURKEY
2D	*T. durum*	KIZILTAN	TURKEY
3D	*T. durum*	ALTINTAS	TURKEY
4D	*T. durum*	DUMLUPINAR	TURKEY
5D	*T. durum*	YELKEN	TURKEY
6D	*T. durum*	KUNDURU	TURKEY
1T	*Ae. tauschii*	MVGB605	HUNGARY
2T	*Ae. tauschii*	MVGB1323	HUNGARY
3T	*Ae. tauschii*	MVGB589	HUNGARY
4T	*Ae. tauschii*	CIMMYT.224	CIMMYT
5T**	*Ae. tauschii*	CIMMYT.369	CIMMYT
6T	*Ae. tauschii*	CIMMYT.372	CIMMYT
7T	*Ae. tauschii*	CIMMYT.458	CIMMYT
8T**	*Ae. tauschii*	CIMMYT.895	CIMMYT

** *Few germinated plant (1 plant for 5T and 2 plants for 8T)*

Table 2. Sowing date, start to vernalization date and transfer to greenhouse of experimental plants and their plant numbers (in 2015).

Weeks	Sowing date	Start of vernalization	Transfer to greenhouse	Planted seeds per variety or access	Total
1	07. Sept.	16. Sept.	04. Nov.	6 durum varieties (8 seeds/var)	48
2	14. Sept.	25. Sept.	11. Nov.	6 durum varieties (8 seeds/var)	48
3	21. Sept.	02. Oct.	18. Nov.	6 durum varieties (8 seeds/var)	48
4	28. Sept.	12. Oct.	25. Nov.	6 durum varieties (8 seeds/var), 5 *Ae.tauschii*, CIMMYT (∑55 seeds), 3 *Ae. tauschii*, MVGB (10 seeds/acc)	123
5	05. Oct.	19. Oct.	2. Dec.	6 durum variety (8 seeds/var), 3 *Ae. tauschii*, MVGB (10 seeds/acc)	78
6	12. Oct.	22. Oct.	9. Dec.	6 durum variety (8 seeds/var)	48
Total pots (There was one plant per pot)					**393**

CIMMYT: Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center)

MVGB: Martonvaser Gene Bank of Agricultural Institute of the Centre for Agricultural Research of the Hungarian Academy of Sciences

Table 3. Numbers of emasculated and pollinated spikes, total flowers and flowers per spike in durum wheat

Cross	Number of		
	Emasculated and Pollinated Spikes (EPS)*	Total Flowers (TF)	Flowers per Spike (TF/EPS)
1D/1T	2	59	29.5
1D/2T	5	161	32.2
1D/3T	8	236	29.5
1D/4T	2	61	30.5
1D/6T	4	133	33.3
1D/7T	2	59	29.5
2D/1T	6	183	30.5
2D/2T	5	140	28.0
2D/3T	11	301	27.4
2D/4T	-	-	-
2D/6T	6	174	29.0
2D/7T	4	121	30.3
3D/1T	5	133	26.6
3D/2T	4	114	28.5
3D/3T	5	154	30.8
3D/4T	4	116	29.0
3D/6T	3	92	30.7
3D/7T	5	136	27.2
4D/1T	6	150	25.0
4D/2T	4	126	31.5
4D/3T	7	187	26.7
4D/4T	-	-	-
4D/6T	3	82	27.3
4D/7T	5	134	26.8
5D/1T	4	130	32.5
5D/2T	6	166	27.7
5D/3T	15	384	25.6
5D/4T	8	222	27.8
5D/6T	3	87	29.0
5D/7T	4	112	28.0
6D/1T	5	174	34.8
6D/2T	4	138	34.5
6D/3T	7	257	36.7
6D/4T	5	188	37.6
6D/6T	3	102	34.0
6D/7T	1	37	37.0
Total	**171**	**5049**	**-**
Mean	**5**	**-**	**30.1**

* *We had 84 pollinated spikes at the end of the first term and we have made more 87 pollination in the first days of the second term*

Table 4. Number and ratio of rescued embryos (RE), germinated embryos in petri-dish medium (GE), plants in tube (PT) and plants in soil (PS) of *Triticum durum* and *Aegilops tauschii genotypes* in all of crosses

		RE		GE		PT		PS	
	Genotype	**Total**	**%**	**Total**	**%**	**Total**	**%**	**Total**	**%**
Triticum durum	1D	30	18.3	15	21.4	11	19.6	11	26.8
	2D	31	18.9	15	21.4	9	16.1	5	12.2
	3D	20	12.2	6	8.6	4	7.1	3	7.3
	4D	26	15.9	5	7.1	4	7.1	2	4.9
	5D	48	29.3	28	40.0	27	48.2	19	46.3
	6D	9	5.5	1	1.4	1	1.8	1	2.4
	Total	**164**	**100**	**70**	**100**	**56**	**100**	**41**	**100**
Aegilops tauschii	1T	1T	17	10.4	4	5.7	4	7.1	4
	2T	2T	19	11.6	6	8.6	5	8.9	5
	3T	3T	67	40.9	43	61.4	33	58.9	22
	4T	4T	18	11.0	7	10.0	7	12.5	6
	6T	6T	21	12.8	3	4.3	3	5.4	2
	7T	7T	22	13.4	7	10.0	4	7.1	2
	Total	**164**	**100**	**70**	**100**	**56**	**100**	**41**	**100**

Table 5. Number of rescued embryos (RE), germinated embryos in petri-dish medium (GE), plants in tube (PT) and plants in soil (PS) of all combinations

Cross	Number of			
	RE	GE	PT	PS
1D/1T	-	-	-	-
1D/2T	11	6	5	5
1D/3T	13	8	5	5
1D/4T	-	-	-	-
1D/6T	6	1	1	1
1D/7T	-	-	-	-
2D/1T	8	4	4	4
2D/2T	-	-	-	-
2D/3T	16	9	3	-
2D/6T	7	2	2	1
2D/7T				
3D/1T	-	-	-	-
3D/2T	-	-	-	-
3D/3T	7	3	2	1
3D/4T	5	2	2	2
3D/6T	-	-	-	-
3D/7T	8	1	-	-
4D/1T	9	-	-	-
4D/2T	4	-	-	-
4D/3T	7	4	4	2
4D/6T	-	-	-	-
4D/7T	6	1	-	-
5D/1T	-	-	-	-
5D/2T	-	-	-	-
5D/3T	24	19	19	14
5D/4T	8	4	4	3
5D/6T	8	-	-	-
5D/7T	8	5	4	2
6D/1T	-	-	-	-
6D/2T	4	-	-	-
6D/3T	-	-	-	-
6D/4T	5	1	1	1
6D/6T	-	-	-	-
6D/7T	-	-	-	-
Total	**164**	**70**	**56**	**41**
[1]Mean 1	**9**	**5**	**4**	**3**
[2]Mean 2	**5**	**-**	**-**	**-**

[1]Mean 1: average of values; [2]Mean 2: average of all of crosses

Table 6. Calculated values of embryo development ability (EDA), embryo germination ability (EGA), vegetative growth ability in tube (VGA) and soil adaptation ability (SAA) of all combinations

Cross	EDA	EGA	VGA	SAA
1D/1T	0.0	-	-	-
1D/2T	6.8	54.5	45.5	100.0
1D/3T	5.5	61.5	38.5	100.0
1D/4T	0.0	-	-	-
1D/6T	4.5	16.7	16.7	100.0
1D/7T	0.0	-	-	-
2D/1T	4.4	50.0	50.0	100.0
2D/2T	0.0	-	-	-
2D/3T	5.3	56.3	18.8	0.0
2D/6T	4.0	28.6	28.6	50.0
2D/7T	0.0	-	-	-
3D/1T	0.0	-	-	-
3D/2T	0.0	-	-	-
3D/3T	4.5	42.9	28.6	50.0
3D/4T	4.3	40.0	40.0	100.0
3D/6T	0.0	-	-	-
3D/7T	5.9	12.5	0.0	0.0
4D/1T	6.0	0.0	0.0	-
4D/2T	3.2	0.0	0.0	-
4D/3T	3.7	57.1	57.1	50.0
4D/6T	0.0	-	-	-
4D/7T	4.5	16.7	0.0	0.0
5D/1T	0.0	-	-	-
5D/2T	0.0	-	-	-
5D/3T	6.3	79.2	79.2	73.7
5D/4T	3.6	50.0	50.0	75.0
5D/6T	9.2	0.0	0.0	-
5D/7T	7.1	62.5	50.0	50.0
6D/1T	0.0	-	-	-
6D/2T	2.9	0.0	0.0	-
6D/3T	0.0	-	-	-
6D/4T	2.7	20.0	20.0	100.0
6D/6T	0.0	-	-	-
6D/7T	0.0	-	-	-
General	**3.2**	**42.7**	**34.1**	**73.2**

Figure 1. Durum wheats were germinated in jiffy pod (A) and transferred to pots in greenhouse (B)

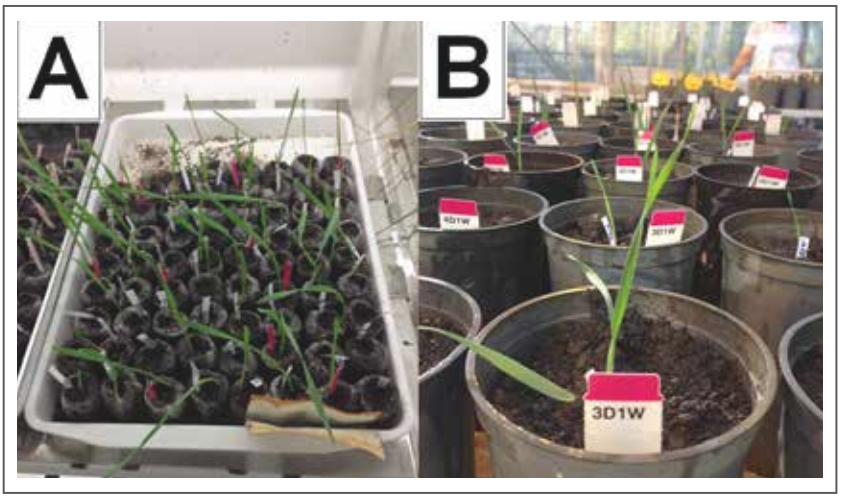

Figure 2. The spikes are ready for sterilization (A) and sterilization processes (B, C)

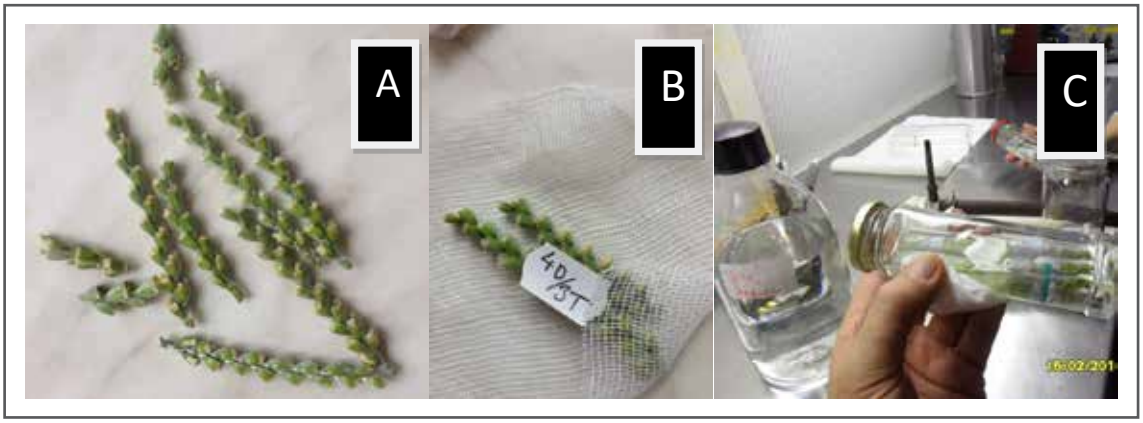

Figure 3. Self-pollinated seeds (on the left in A) in durum wheat; and cross-pollinated durum wheat seeds with male parent *Ae. tauschii* (on the right in A); a successful inter-specific hybridized seed with embryo (C and D); 2.4-D caused normal looking seed (B), but seed had no embryo (C and D)

Figure 4. Germinated inter-specific hybridized seeds in B-5 medium in petrie dish (A), transferring of germinated seeds from petrie dish to tube after three weeks (B), developments of shoot and root of haploid plants in the tubes (C and D)

Figure 5. Some fungal contaminations in B-5 mediums in petrie dish (A) and tube (B)

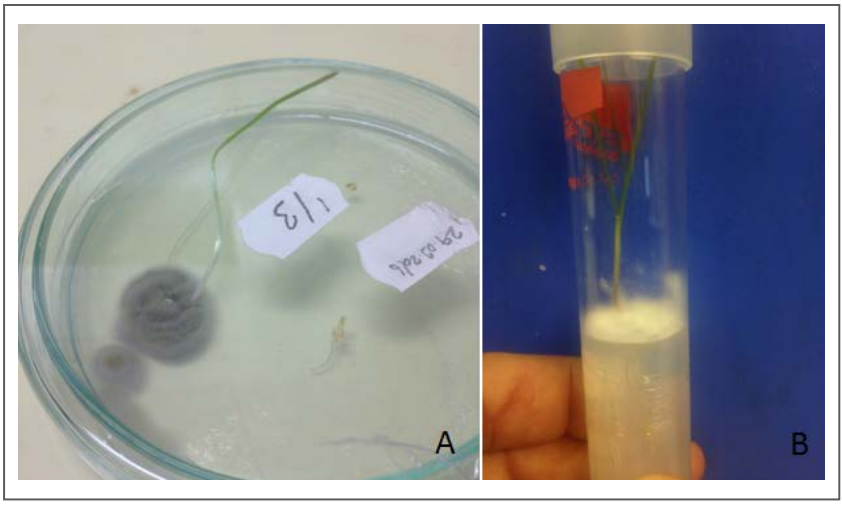

Figure 6. Transfer well formed haploid plants from B-5 medium into soil after vernalization (A and B), covering them with plastic wrap to prevent moisture loss during one week (C)

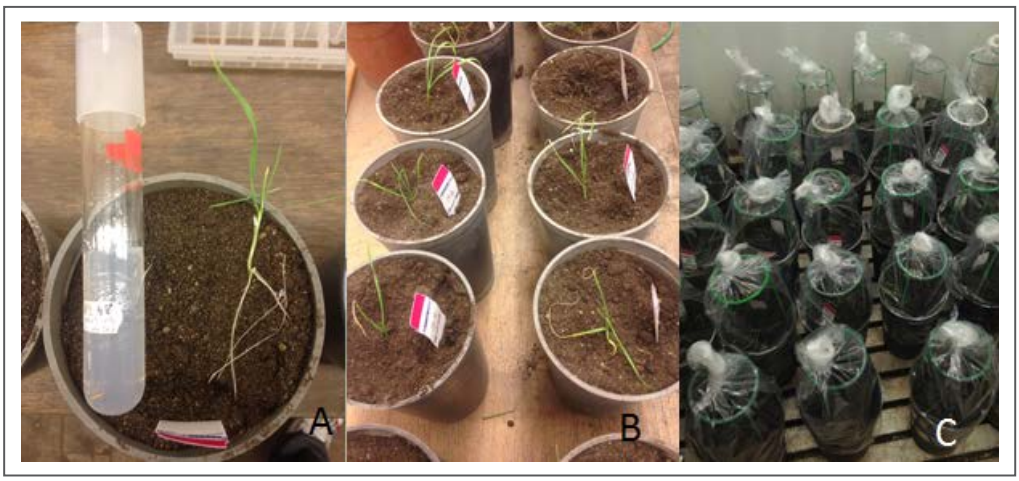

Figure 7. Prepared haploid plants for colchicine treatment (A), washing of roots (B) and cuting of roots (C)

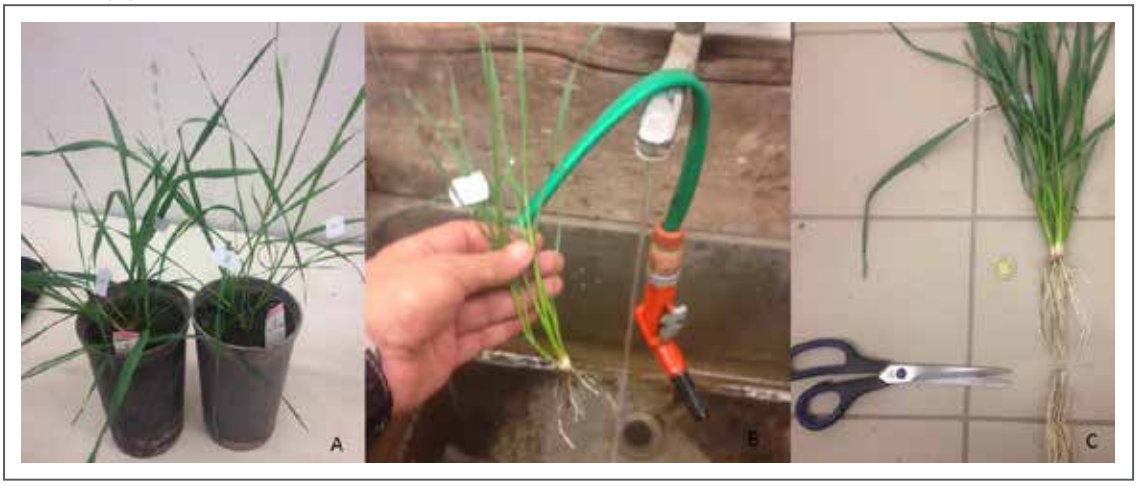

Figure 8. Colchicine treatment to haploid plants (A) and washing of colchicine treated parts of plants under tap water (B)

Figure 9. Cuting of leaves ofcolchicine treated plants (A). Plant transfer into soil (B). Plants in phytotron (C)

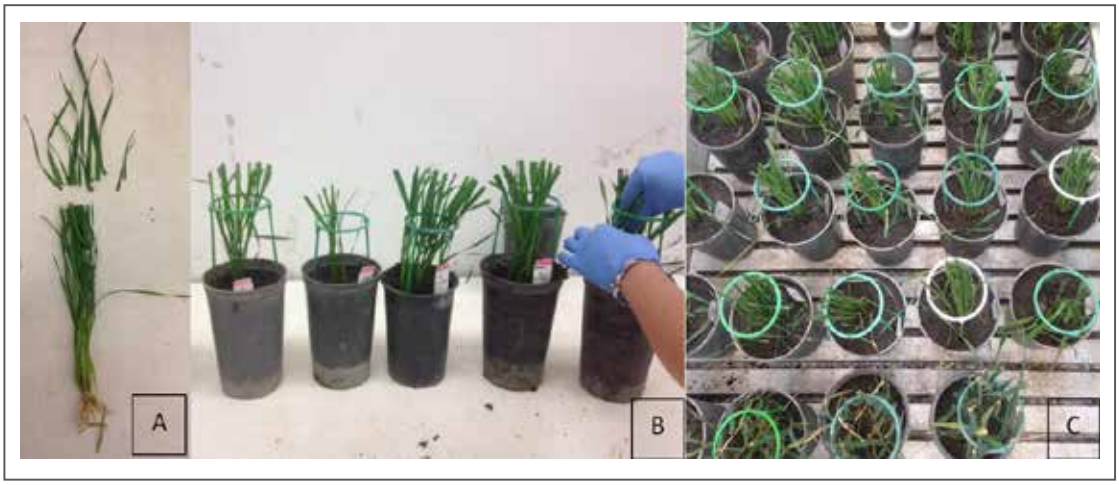

Figure 10. Some deformations and damages on plant after colchicine treatment

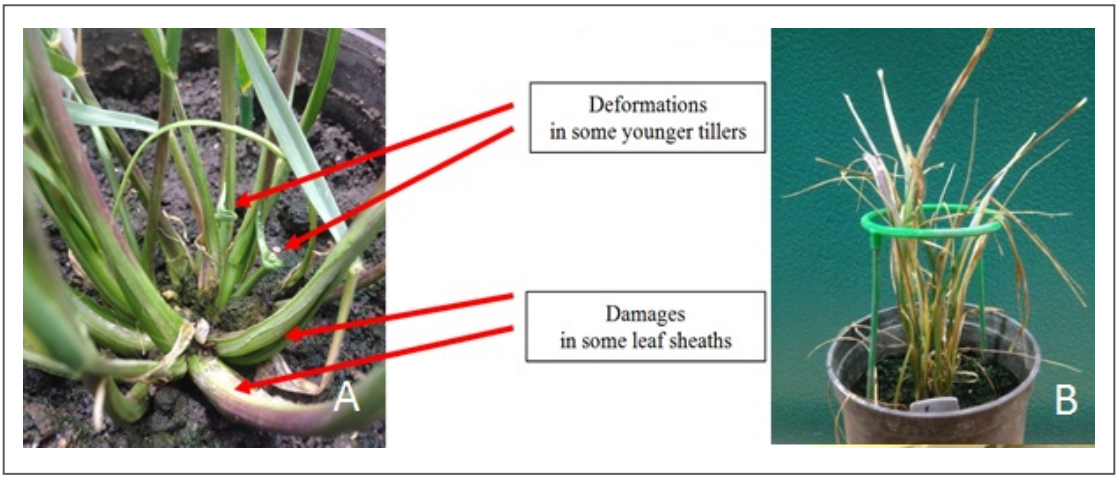

Figure 11. Shooting younger tillers after the death of older tillers

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