UDC 575.633.11
https://doi.org/10.2298/GENSR2001245T
Original scientific paper

CHEMICALLY INDUCED MALE STERILITY IN COMMON WHEAT MEDIATED BY *AEGILOPS* CYTOPLASM

Primož TITAN[1,2], Jernej ISKRA[3], Koji MURAI[4], Vladimir MEGLIČ[5*]

[1]RGA, Research Genetics and Agrochemistry, Ltd, Murska Sobota, Slovenia
[2] University of Maribor, Faculty of Agriculture and Life Sciences, Maribor, Slovenia
[3]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia
[4]Fukui Prefectural University, Fukui, Japan
[5] Agricultural Institute of Slovenia, Ljubljana, Slovenia

Titan P., J. Iskra, K. Murai, V. Meglič (2020). *Chemically induced male sterility in common wheat mediated by* A*egilops cytoplasm* Genetika, Vol 52, No.1, 245-256.

Several approaches have been proposed, in the past, for the development of hybrid wheat varieties. Nevertheless, common wheat hybrids today account for less than 1% of the world's wheat production. The reason for the limited success of hybrid wheat varieties is the fact that to date, a simple and efficient system for the production of hybrid seed has not been developed. A two-line system using photoperiod-sensitive cytoplasmic male sterility (PCMS) caused by *Aegilops crassa* Boiss. cytoplasm seems to be very promising way to produce hybrid wheat seed on large scale. Non-desirable side effects of PCMS system are the unreliable sterility of the male sterile lines under different latitudes. Therefore, we tried to connect male sterility system based on *Ae. crassa* cytoplasm with chemical induction of male sterility. The presented results reveal that the majority of male sterility genes are conserved in the *Ae. crassa* cytoplasm and that their effects can be promoted by chemical signals, such as synthetic auxins (e.g., 2,4-Dichlorophenoxyacetic acid). Our research work represents the development of a novel male sterility system which is controlled by chemical signals (e.g., synthetic auxins and their pro-herbicide analogues) and mediated by *Ae. crassa* cytoplasm.

Keywords: *Aegilops* cytoplasm, azetidine-3-carboxylic acid, hybrid wheat, male sterility, 2,4-Dichlorophenoxyacetic acid

INTRODUCTION

The development of hybrid wheat has a long history. This history can be placed into the period between 1970s and 1990s and a revival in the last couple of years. In both periods, several approaches for the induction of male sterility in the female component of the hybrid variety were investigated. Initial approaches were based on the use of cytoplasmic-genetic male sterility

Corresponding authors: Vladimir Meglič, Crop science department, Agricultural institute of Slovenia, Ljubljana, Slovenia, Phone +386 (0)1 280 51 80, E-mail: vladimir.meglic@kis.si

(CMS) and chemically-interfered male sterility (LONGIN *et al.,* 2012; LONGIN *et al.,* 2014; MUHLEISEN *et al.,* 2014). Recently, transgenic approaches for the induction of male sterility were also proposed. Examples of such approaches are split gene approach (system based on barnase/barstar gene) and a system based on gene for a phospholipid-binding protein (glycosylphosphatidylinositol (GPI)-anchored lipid transfer protein, *Ms1* (*Male sterility 1*) gene) (TUCKER *et al.,* 2017; WANG *et al.,* 2017). Nowadays, the majority of hybrid wheat varieties are still developed using the chemically-interfered male sterility (WHITFORD *et al.,* 2013).

Chemically-interfered male sterility is based on the application of chemical hybridizing agent (CHA). The chemical hybridizing agent is an established term for a chemical, which has a distinct selective effect on microsporogenesis or the development of viable pollen in the female component of a hybrid variety. The connection between selective effect of chemicals on the plant metabolism in association with induction of male sterility and production of hybrid seed could be traced to as early as 1957, when the production of hybrid cotton seed with the use of the chemical FW-450 (active substance α,β-dichloro isobutyrate) was described (JOHNSON and SCHMIDT, 1968).

Chemicals with known effectiveness as chemical hybridizing agents for the hybrid wheat seed production belong to the group of pollen germination inhibitors, like the active substance azetidine-3-carboxylic acid (agent WL 84811) and microsporogenesis inhibitors. Examples of microsporogenesis inhibitors are active substance fenridazon (agent HYBREX®), clofencet (agents GENESIS® and SQ-1) and sintofen (agent CROISOR® 100) (SINGH *et al.,* 2014). In our previous research work we have investigated also ethyl oxanilates used as microsporogenesis inhibitors (ISKRA *et al*., 2013). Similar to the Indian authors (CHAKRABORTY and DEVAKUMAR, 2006), we have tested this chemicals in the pre-meiotic stage of wheat and confirmed their impact on the male sterility induction. The effectiveness of CHA is often hindered by the environmental factors and by the interaction with the genotype. Several phytotoxic effects like female sterility, narrow window of application, toxic residues in soil and F1 seed material, mutagenic action and high production costs made the commercial use of CHA very risky. These facts are also among the main reasons why Croisor® 100 is one of the few chemical hybridizing agents in the world still in the use today (WHITFORD *et al.,* 2013). In addition to known CHAs, the chemical fertility control systems can also be based on the conversion of the non-toxic herbicide analogues like D-glufosinate into their phytotoxic analogues (L-glufosinate). In this case the conversion of non-toxic analogue to its toxic version is based on the engineered enzyme (D-amino acid oxidase – DAAO) and has therefore a very limited value for the commercial use (HAWKES *et al.,* 2011).

Cytoplasmic male sterility (CMS) and genetic male sterility (GMS) systems can be temperature and/or photoperiod sensitive (WHITFORD *et al.,* 2013). Temperature and/or photoperiod sensitive male sterility is most commonly associated with two types of cytoplasm: K type (donor of cytoplasm is *Aegilops kotschyi*) and D^2 type (donor of cytoplasm is *Ae. crassa*) (MENG *et al.,* 2016; MURAI *et al.,* 2008; MURAI *et al.,* 2016). K type of cytoplasm is associated with thermo-sensitive cytoplasmic male-sterility (TCMS) and is mainly investigated in association with the development of commercial wheat hybrids in China (MENG *et al.,* 2016; ZHANG *et al.,* 2001). In this paper, we refer to the conditional male sterility which is caused by the sensitiveness of *Ae. crassa* cytoplasm to long day conditions (so-called 'photoperiod-

sensitive cytoplasmic male sterility' – PCMS). The PCMS system has been developed for hybrid wheat breeding in Japan, where hybrid seeds are obtained in spring-sown conditions in Hokkaido, and hybrid varieties are planted in middle to south part of Honshu. The fertility of hybrid varieties is restored by short-day conditions and *Rf* genes (MURAI, 2001a; MURAI, 2001b). To apply the PCMS system to the European climate conditions, we need an activator to gain complete sterility of the PCMS lines and complete fertility restoration of hybrid varieties. Therefore, we tried to connect male sterility system based on *Ae. crassa* cytoplasm with chemical induction of male sterility using the azetidine-3-carboxylic acid (A3C). The present results revealed that the majority of male sterility genes are conserved in *Ae. crassa* cytoplasm and that their effects can be further promoted by chemical signals such as synthetic auxins (e.g., 2,4-dichlorophenoxyacetic acid (2,4-D)). Our research work represents the development of a novel male sterility system which is controlled by a chemical signals (e.g., synthetic auxins and their pro-herbicide analogues) and is mediated by *Ae. crassa* cytoplasm. The research and breeding work described represents the development of conditional chemical male sterility system on a non-GM basis.

MATERIALS AND METHODS

Plant materials

The work was carried out at the experimental field of the RGA company, Research Genetics and Agrochemistry, Ltd. located at Krog near Murska Sobota (latidude 46°37'22.58"N, longitude 16°7'44.10"E), NE Slovenia. To introduce the *Aegilops* genome into modern wheat germplasm, we carried out 15 crosses in 2014 and 44 crosses in 2015. The crosses were carried out between Japanese alloplasmic lines with *Ae. crassa* Boiss. Cytoplasm and CIMMYT hexaploid synthetic lines (*Triticum durum* Desf. × *Ae. squarrosa* L.) with modern common wheat varieties (*T. aestivum* L.). To check the impact of the replacement of *Ae. crassa* chromosomes with additional *T. aestivum* chromosomes on the male sterility induction we carried out backcrosses (BC2). Backcrosses involved 15 crosses which were carried out in 2014. Undesirable side effects of *Ae. crassa* cytoplasm under long day conditions are inter alia pistillody (homeothic transformation of stamens into pistil-like structures) (MURAI *et al.*, 2002). All materials were sown in the autumn conditions. The crosses and parental lines used in our research work are presented in the Table 1. Crosses were performed by hand emasculation and controlled pollination, and successive offspring generations were selected following the pedigree method. For selection of breeding materials, the following descriptors were used: plant height, spike length, seed set under long day and short day conditions and heading time.

Table 1. List of F_3 generations and parental lines used in the study

F3 generation	Parents		Seed set in F_2 generation
	Female	Male (*T. aestivum*)	
CIGM90.865/101	CIGM90.865[a]	Bezostaya 1	43
CIGM90.865/107	CIGM90.865[a]	Fortunato	39
CIGM90.865/88	CIGM90.865[a]	Chinese spring	47
CIGM90.865/99	CIGM90.865[a]	Norin 61	53
CIGM92.1682/101	CIGM92.1682[a]	Bezostaya 1	44
CIGM92.1682/107	CIGM92.1682[a]	Fortunato	39

CIGM92.1682/88	CIGM92.1682[a]	Chinese spring	36
CIGM92.1682/99	CIGM92.1682[a]	Norin 61	49
CIGM92.1682/1	CIGM92.1682[a]	Alixan	44
PCMS 2013.1/101	PCMS 2013.1[b]	Bezostaya 1	46
PCMS 2013.1/107	PCMS 2013.1[b]	Fortunato	27
PCMS 2013.1/88	PCMS 2013.1[b]	Chinese spring	31
PCMS 2013.1/99	PCMS 2013.1[b]	Norin 61	22
PCMS 2013.1/67	PCMS 2013.1[b]	Felix	17
PCMS 2013.3/101	PCMS 2013.3[b]	Bezostaya 1	38
PCMS 2013.3/107	PCMS 2013.3[b]	Fortunato	19
PCMS 2013.3/88	PCMS 2013.3[b]	Chinese spring	21
PCMS 2013.3/99	PCMS 2013.3[b]	Norin 61	22
PCMS 2013.3/67	PCMS 2013.3[b]	Felix	41
PCMS 2013.5/101	PCMS 2013.5[b]	Bezostaya 1	15
PCMS 2013.5/107	PCMS 2013.5[b]	Fortunato	13
PCMS 2013.5/88	PCMS 2013.5[b]	Chinese spring	27
PCMS 2013.5/99	PCMS 2013.5[b]	Norin 61	21
PCMS 2013.5/67	PCMS 2013.5[b]	Felix	11
RGPCMS 14.14/101	RGPCMS 14.14[b]	Bezostaya 1	40
RGPCMS 14.14/107	RGPCMS 14.14[b]	Fortunato	17
RGPCMS 14.14/88	RGPCMS 14.14[b]	Chinese spring	18
RGPCMS 14.14/99	RGPCMS 14.14[b]	Norin 61	14
RGPCMS 14.14/67	RGPCMS 14.14[b]	Felix	33
PCMS 2013.1//101(BC2)	PCMS 2013.1/101[c]	Bezostaya 1	37
PCMS 2013.1//107(BC2)	PCMS 2013.1/107[c]	Fortunato	12
PCMS 2013.1//88(BC2)	PCMS 2013.1/88[c]	Chinese spring	6
PCMS 2013.1//99(BC2)	PCMS 2013.1/99[c]	Norin 61	18
PCMS 2013.1//67(BC2)	PCMS 2013.1/67[c]	Felix	22
PCMS 2013.3//101(BC2)	PCMS 2013.3/101[c]	Bezostaya 1	33
PCMS 2013.3//107(BC2)	PCMS 2013.3/107[c]	Fortunato	11
PCMS 2013.3//88(BC2)	PCMS 2013.3/88[c]	Chinese spring	15
PCMS 2013.3//99(BC2)	PCMS 2013.3/99[c]	Norin 61	20
PCMS 2013.3//67(BC2)	PCMS 2013.3/67[c]	Felix	44
RGPCMS 14.14//101(BC2)	RGPCMS 14.14/101[c]	Bezostaya 1	47
RGPCMS 14.14//107(BC2)	RGPCMS 14.14/107[c]	Fortunato	51
RGPCMS 14.14//88(BC2)	RGPCMS 14.14/88[c]	Chinese spring	47
RGPCMS 14.14//99(BC2)	RGPCMS 14.14/99[c]	Norin 61	50
RGPCMS 14.14//67(BC2)	RGPCMS 14.14/67[c]	Felix	53

[a]*Triticum durum* × *Aegilops squarrosa*; [b]*Aegilops crassa* × *Triticum durum*;[c](*Aegilops crassa* × *Triticum durum*) × *T. aestivum*

Preparation and application of chemicals

Azetidine-3-carboxylic acid – A3C was obtained from commercial sources and was used as received. 2,4-Dichlorophenoxyacetic acid – 2,4-D was obtained from commercial sources as herbicide Mustang 306 SE (45,242 % 2,4-D 2-ethylhexyl ester).

The chemical induction of male sterility was tested on F_3 progenies selected from the F_2 generations with the highest seed set (in total, 19 F_3 generations). For this purpose, 30 spikes per each F_2 generation in 2017 were randomly collected and the average number of seeds per spike was determined. To induce the male sterility in selected F_3 generations, we used the synthetic auxin 2,4-dichlorophenoxyacetic acid, azetidine-3-carboxylic acid as a chemical with known effect as chemical hybridizing agent and the combination of both of them. F_3 generations were sown in 6 meter long twin rows (row to row distance was kept at 25 cm) in October 2017. The chemicals were applied in the spring of 2018 on 0.5 meter wide strips which were perpendicular to the direction of the twin rows (Figure 1). Spray suspensions were applied in morning hours when the air temperature exceeded 10°C and the relative air humidity was at least 60 %. For the application we used ground application equipment with low-pressure and low drift flat fan nozzles (Lechler nozzle 110-04). 2,4-D was applied in the form of herbicide with the commercial name Mustang 306 SE (45,242 % 2,4-D 2-EHE) in the doses 0,75 and 1 liter per hectare when the wheat plants reached the phenophase 31/32 according to the BBCH classification (BBCH Monograph, 2018). A3C was applied together with the surfactant Trend 90 (900 g/L isodecyl alcohol ethoxylate) in the dose of 400 and 800 grams per hectare when the wheat plants reached the BBCH 37 (flag leaf just visible, still rolled). The consumption of spray suspension was kept at 400 L per hectare.

Figure 1. F_3 generations after the application of experimental chemicals (culminating spikes are marked with red line)

Data collection

The effectiveness of tested chemicals on male sterility induction was estimated using the calculation of male sterility percentage, plant height and spike length. Plant height was measured when wheat plants reached the phenophase 69 according to the BBCH classification (end of flowering, flowering has terminated in all spikelets but some dehydrated anthers may remain). Spike length was determined on fully mature plants, phenophase 89 according to the BBCH classification (BBCH Monograph, 2018). The percentage of male sterility was determined on the basis of seed set in spikes of treated and untreated (control) plants. Therefore, we isolated ten spikes for each treatment. Wheat spikes were isolated with white paper bags at the BBCH 58/59 (the whole spike is visible). To avoid any statistical failures, we isolated also 10 spikes of control plants. The completed counting of grains of treated and control plants were followed by the calculation of the percentage of male sterility in accordance to the following formula:

$$\% \text{ of male sterility} = ((S_C – S_T) / S_C) \times 100$$

S_T: Number of grains in isolated spikes of treated plants.
S_C: Number of grains in isolated spikes of un-treated (control) plants.

Test of significance

Statistical analysis was performed using the statistical package STATGRAPHICS Centurion XVI. Significance was tested according to Duncan's multiple range test at $p \leq 0.05$.

RESULTS

Influence of tested chemicals on male sterility, plant height and spike length

Analysis of variance revealed significant differences for all observed parameters (Table 2). This means that for selected F_3 generations and chemicals, a statistically significant impact on the male sterility, plant height and spike length was documented. Also for the interaction F_3 generation × chemical, significant impact on all observed parameters was determined. Azetidine-3-carboxylic acid expressed high impact on the male sterility induction, especially in the combination with 2,4-Dichlorophenoxyacetic acid. 2,4-D had also a positive impact on the plant height. The highest dose of 2-4 D resulted with the statistically significant plant height. Additional application of the A3C resulted with the reduction of plant height. On the other hand, the application of 2,4-D had a negative impact on the spike length. The increase of 2,4-D dose resulted in a significantly shorter spike length. The addition of A3C to 2,4-D did not have significant impact on the spike length.

Table 2. Analysis of mean values for the observed parameters

	Male sterility (%)	Plant height (cm)	Spike length (cm)
	Average values ± SE		
F_3 generation			
CIGM90.865/101	$12,31 \pm 13,88^{k}$	$75,91 \pm 2,45^{de}$	$16,29 \pm 0,66^{c}$
CIGM90.865/107	$13,83 \pm 16,07^{k}$	$85,06 \pm 3,03^{b}$	$15,44 \pm 0,78^{e}$
CIGM90.865/88	$10,04 \pm 10,64^{l}$	$85,59 \pm 2,61^{a}$	$14,69 \pm 0,59^{f}$

CIGM90.865/99	35,66 ± 30,97^{h}	75,78 ± 2,02^{e}	15,95 ± 0,91^{d}
CIGM92.1682/1	40,14 ± 33,59^{g}	84,51 ± 3,42^{c}	13,71 ± 0,70^{g}
CIGM92.1682/101	16,79 ± 21,55^{j}	85,3 ± 2,74^{ab}	17,37 ± 1,31^{a}
CIGM92.1682/107	16,83 ± 21,52^{j}	85,48 ± 2,51^{ab}	10,98 ± 0,66^{h}
CIGM92.1682/88	16,30 ± 19,27^{j}	76,31 ± 2,81^{ab}	14,86 ± 0,78^{f}
CIGM92.1682/99	23,94 ± 25,81^{i}	66,9 ± 2,32^{d}	16,56 ± 0,73^{b}
PCMS 2013.1//101	70,57 ± 35,88^{f}	66,46 ± 2,09^{i}	9,51 ± 0,94^{i}
PCMS 2013.1/101	72,24 ± 35,22^{e}	68,6 ± 2,176^{i}	9,39 ± 0,92^{i}
PCMS 2013.3//101	73,57 ± 32,25^{de}	69,09 ± 1,81^{g}	8,65 ± 0,60^{jk}
PCMS 2013.3//67	74,84 ± 32,58^{cd}	67,91 ± 1,18^{fg}	7,85 ± 0,76^{no}
PCMS 2013.3/101	78,59 ± 33,41^{a}	69,53 ± 1,98^{h}	8,54 ± 0,52^{kl}
PCMS 2013.3/67	76,91 ± 32,98^{b}	66,58 ± 1,16^{f}	7,78 ± 0,64^{n}
RGPCMS 14.14//101	76,37 ± 33,23^{bc}	67,65 ± 2,23^{i}	8,38 ± 0,90^{j}
RGPCMS 14.14//67	73,17 ± 32,01^{e}	66,43 ± 2,11^{h}	8,23 ± 0,76^{m}
RGPCMS 14.14/101	77,53 ± 33,00^{ab}	67,75 ± 2,26^{i}	8,78 ± 1,08^{lm}
RGPCMS 14.14/67	76,57 ± 33,16^{b}	74,54 ± 2,14^{h}	7,99 ± 0,61^{n}
Chemicals*			
2,4D 0,75	32,76 ± 31,75^{f}	75,59 ± 7,33^{b}	11,67 ± 3,59^{c}
2,4D 0,75 + A3C 400	62,52 ± 38,98^{c}	73,79 ± 7,82^{d}	11,56 ± 3,65^{cd}
2,4D 1	47,18 ± 40,56^{e}	77,07 ± 7,94^{a}	11,30 ± 3,68^{f}
2,4D 1 + A3C 800	77,66 ± 25,82^{a}	74,06 ± 8,88^{d}	11,34 ± 3,72e^{f}
A3C 400	52,43 ± 36,38^{d}	74,52 ± 8,34^{c}	11,63 ± 3,61^{c}
A3C 800	71,83 ± 30,98^{b}	74,43 ± 9,33^{c}	11,46 ± 3,78^{de}
Trend 90	0,55 ± 1,03^{g}	73,01 ± 7,73^{d}	11,86 ± 3,43^{b}
(control) plants		73,85 ± 7,45^{d}	12,21 ± 3,52^{a}

Mean values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test at $p \leq 0.05$.

* 2,4D 0,75 (0,75 L ha^{-1} herbicide Mustang 306 SE); 2,4D 0,75 + A3C 400 (0,75 L ha^{-1} herbicide Mustang 306 SE + 400 g ha^{-1} azetidine-3-carboxylic acid + surfactant Trend 90 (0,1 %)); 2,4D 1 (1 L ha^{-1} herbicide Mustang 306 SE); 2,4D 1 + A3C 800 (1 L ha^{-1} herbicide Mustang 306 SE + 800 g ha^{-1} azetidine-3-carboxylic acid + surfactant Trend 90 (0,1 %)); A3C 400 (400 g ha^{-1} azetidine-3-carboxylic acid + surfactant Trend 90 (0,1 %)); A3C 800 (800 g ha^{-1} azetidine-3-carboxylic acid + surfactant Trend 90 (0,1 %)); Trend 90 (surfactant: 900 g/L isodecyl alcohol ethoxylate)

Genetic background and male sterility

F_3 generations with the *Aegilops crassa* cytoplasm expressed a significantly higher percentage of male sterility in comparison to other F_3 generations. Highest percentage of male sterility was achieved in F_3 generations PCMS 2013.3/101 and RGPCMS 14.14/101 (Table 2). Crosses of PCMS materials with common wheat varieties had a negative impact on the male sterility induction. All crosses with common wheat (*Triticum aestivum*) expressed significant lower percentage of male sterility in comparison to their PCMS analogues. The analysis of the interaction between F_3 generation and chemical revealed that there was a positive relationship in terms of male sterility induction between *Ae. crassa* based cytoplasm and 2,4-

Dichlorophenoxyacetic acid (Figure 2). The increase of 2,4-D dose in PCMS accessions resulted with high levels of male sterility. In some cases the highest dose of 2,4-D resulted with a significantly higher percentage of male sterility in comparison to lower dose of Azetidine-3-carboxylic acid which is known CHA.

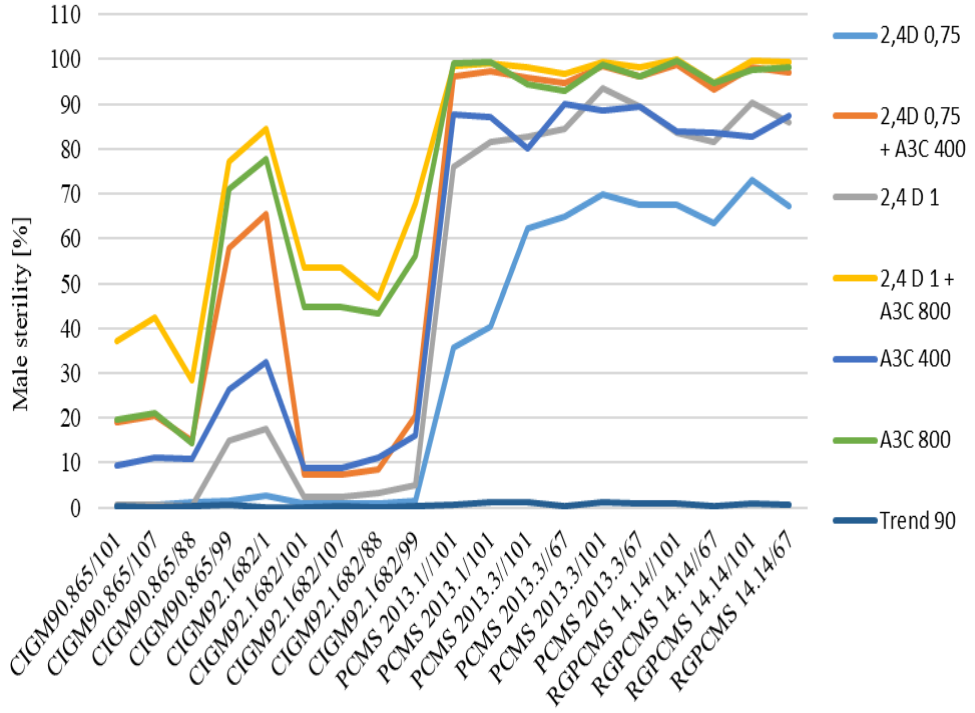

Figure 2. Impact of the interaction F_3 generation × Chemical on the male sterility induction

DISCUSSION

The hybrid wheat breeding using the *Aegilops* cytoplasm is most often associated with cytoplasmic male sterility systems that are conditioned by environmental factors, particularly temperature and/or photoperiod (TANIO *et al.*, 2005; MURAI *et al.*, 2008). Photoperiod-sensitive cytoplasmic male sterile (PCMS) lines have already been developed (MURAI *et al.*, 2016). To achieve complete male sterility expression under different latitudes, we have tried to associate male sterility system based on *Ae. crassa* cytoplasm with chemical induction of male sterility. Chemically inducible systems, that activate or inactivate gene expression, have many potential applications in plant breeding. The precise timing and control of gene expression are important aspects of chemically inducible systems. Several systems have been developed and used to analyze gene function, marker-free plant transformation, site-specific DNA excision, activation

tagging, conditional genetic complementation, and restoration of male fertility. In our study, we investigated the possibility for the expression of *Aegilops* male sterility genes linked with the chemical signals in the form of synthetic auxins and their pro-herbicide analogues. Therefore, breeding materials were developed that were carriers of the *Aegilops* germplasm and had superior properties in terms of fertility (high seed sets) under long and short day conditions.

The interaction between F_3 generation and chemical revealed that the majority of male sterility genes were conserved in the *Ae. crassa* cytoplasm and they could be promoted by chemical signals, such as synthetic auxins (e.g.. 2,4-Dichlorophenoxyacetic acid). However, some CIMMYT hexaploid synthetic lines (*Triticum durum* × *Aegilops squarrosa*) also reacted on the application of 2,4-D with the increase of male sterility percentage. Taking into the account that the replacement of *Ae. crassa* and *T. durum* chromosomes with *T. aestivum* chromosomes had a negative impact on the male sterility induction, we can assume that some *Aegilops* species are carriers of the male sterility genes which are located in the cytoplasm and nucleus. This could mean that *Aegilops* genes involved in the male sterility expression can be controlled with chemical signals, but only on the cell level.

For the hybrid wheat breeding, it is necessary to introduce the elite common wheat genetic material into *Aegilops* based PCMS materials. Therefore, it is necessary to be focussed on the male sterility genes which are placed in cytoplasm. To confirm the interactions between *Aegilops* gametocidal genes which are inter alia responsible for segregation disorders in wheat-*Aegilops* wide hybridization and synthetic auxin based induction of male sterility, further studies on molecular level are necessary. In any case, the main goal of our future research work is the development of non-GM two-component hybrid wheat system which is not photo- or thermo-sensitive but is driven by controlled chemical signal and mediated by *Aegilops* cytoplasm. In this way, it may be possible to nullify the impact of the geographical location (i.e., latitude) where the hybrid seed production takes place, and this would be the main advantage over the conventional PCMS system. In order to solve the problems described earlier and develop a perfectly reliable and stable cytoplasmic male sterility, it will be necessary to conduct several additional intergeneric *Triticum* × *Aegilops* crosses, combined with systematic backcrosses, self-pollinations, and molecular, morphological and physiological tests. A reliable cytoplasmic male sterility system is crucial for a stable and cost efficient hybrid wheat production. Similarly to other CMS systems, further studies related to the development of effective R (restorer) lines, and general and specific combining ability testing would be very helpful. Undesired interaction between primitive *Triticeae* background (e.g., *Ae. crassa* cytoplasm) and modern common wheat germplasm, however, can represent one of the major drawbacks (e.g. susceptibility on diseases, lodging etc.).

CONCLUSIONS

The main and the final aim of our research is to develop a non-GM, two-component hybrid wheat system which would be stable, not photo- or thermo- sensitive, driven by controlled chemical signals and mediated by *Aegilops* cytoplasm. Numerous crosses involving Japanese alloplasmic lines with *Aegilops crassa* cytoplasm, CIMMYT hexaploid synthetic lines (*Triticum durum* × *Ae. squarrosa*) and modern common wheat varieties (*Triticum aestivum*) were carried in order to develop the basic plant material which would be needed for further crosses and selection. To check the impact of the replacement of *Ae. crassa* chromosomes with

the chromosomes of *T. aestivum* on the male sterility induction, offsprings of 15 crosses were backcrossed. To achieve a complete male sterility expression under different geographical conditions, the *Ae. crassa* based male sterility system was combined with different types of chemical induction of male sterility. The study showed that crosses of PCMS materials with the common wheat varieties had, in general a negative impact on the expression of the male sterility. Regarding individual combinations involving PCMS and common wheat varieties, it is possible to conclude that Bezostaya 1 had generally the most obvious negative effect on the expression of the male sterility. In selected F_3 generations and applied chemicals, significant impacts on the male sterility, plant height and spike length were revealed. A significant impact on these parameters was also determined for the interaction F_3 generation × chemical. When considering the phenotypic variability which depends on genotypic and environmental differences, and their interactions, plant height and spike length cannot be considered, in practice, as reliable parameters for the assessment of the level of male sterility. Regarding the induction of the male sterility, azetidine-3-carboxylic acid (A3C), especially in the combination with 2,4-Dichlorophenoxyacetic acid (2,4-D), was found to be highly efficient. The additional use of A3C resulted in shorter plants, while 2,4-D reduced the spike length. In order to create a fully reliable cytoplasmic male sterility system, it will be necessary to conduct additional intergeneric *Triticum* × *Aegilops* crosses, combined with systematic backcrosses and self-pollinations, and followed by molecular, agro-morphological and physiological tests. It will also be important to test the stability of the resulting sterility system under different aspects of carefully defined environments.

ACKNOWLEDGEMENTS

This research was partly supported by grants from the Slovenian Research Agency, research programme P4-0072 and bilateral project cooperation between Japan and Slovenia BI-JP/16-18-001. We thank CIMMYT (International Maize and Wheat Improvement Center) for providing hexaploid synthetic *Triticum aestivum* lines. We thank also Professor Dr. Ludvik Rozman for the advice regarding the statistical data processing.

Received, July 27th, 2019
Accepted December 18th, 2019

REFERENCES

CHAKRABORTY, K., C., DEVAKUMAR (2006): Evaluation of chemical compounds for induction of male sterility in wheat (*Triticum aestivum* L.). Euphytica, *147*(3): 329-335.

HAWKES, T.R., W., PLINE-SRNIC, R., DALE, E., FRIEND, T., HOLLINSHEAD, P., HOWE, P., THOMPSON, R., VINER, A., GREENLAND (2011): D-Glufosinate as a male sterility agent for hybrid seed production. Plant Biotechnology Journal, *9*: 301-314.

ISKRA, J., P., TITAN, V., MEGLIČ (2013): The effect of fluorine atom on the synthesis and composition of gametocidal ethyl oxanilates. Acta chimica Slovenica, *60*: 561-568.

JOHNSON, V.A., J.W., SCHMIDT (1968): Hybrid wheat. In: Norman AG (ed) Advances in Agronomy, Vol 20, Academic Press, London, pp 199-233.

LONGIN, C.F.H., J., MUHLEISEN, H.P., MAURER, H., ZHANG, M., GOWDA, J.C., REIF (2012): Hybrid breeding in autogamous cereals. Theoretical and Applied Genetics, 125: 1087-1096.

LONGIN, C.F.H., J.C., REIF, T., WURSCHUM (2014): Long-term perspective of hybrid versus line breeding in wheat based

on quantitative genetic theory. TAG, *127*: 1635-1641.

MENG, L., Z., LIU, L., ZHANG, G., HU, X., SONG (2016): Cytological characterization of a thermo sensitive cytoplasmic male-sterile wheat line having K-type cytoplasm of *Aegilops .kotschyi.* Breed. Sci., *66*: 752-761.

MUHLEISEN, J., H-P., PIEPHO, H.P., MAURER, C.F.H., LONGIN, J.C., REIF (2014): Yield stability of hybrids versus lines in wheat, barley, and triticale. TAG, *127*: 309-316.

MURAI, K. (2001a): Genetic effects of an alien cytoplasm on male and female fertility in wheat.Recent Res. Devel. Genet., *1*: 47-54.

MURAI, K. (2001b): Factors responsible for levels of male sterility in photoperiod-sensitive cytoplasmic male sterile (PCMS) wheat lines. Euphytica, *117*: 111-116.

MURAI, K., S., TAKUMI, H., KOGA, Y., OGIHARA (2002): Pistillody, homeothic transformation of stamens into pistil-like structures, caused by nuclear-cytoplasm interaction in wheat. Plant J., 29: 169-181.

MURAI, K., I., TSUTUI, Y., KAWANISHI, S., IKEGUCHI, M., YANAKA, N., ISHIKAWA (2008): Development of photoperiod-sensitive cytoplasmic male sterile (PCMS) wheat lines showing high male sterility under long-day conditions and high seed fertility under short-day conditions. Euphytica, *159*: 315-323.

MURAI, K., H., OHTA, M., KURUSHIMA, N., ISHIKAWA (2016): Photoperiod-sensitive cytoplasmic male sterile elite lines for hybrid wheat breeding, showing high cross-pollination fertility under long-day conditions. Euphytica, *212*: 313-322.

SINGH, S.P., R., SRIVASTAVA, J., KUMAR (2014): Male sterility systems in wheat and opportunities for hybrid wheat development. Acta Physiol. Plant., *37*: 1713.

TANIO, M., K., KATO, N., ISHIKAWA, Y., TAMURA, M., SATO, H., TAKAGI. M., MATSUOKA (2005) Genetic analysis of photoperiod response in wheat and its relation with the earliness of heading in the southwestern part of Japan. Breed. Sci., *55*: 327-334.

TUCKER, E.J., U., BAUMANN, A., KAUIDRI, R., SUCHECKI, M., BAES, M., GARCIA, T. OKADA, C., DONG, Y., WU, A., SANDHU, M., SINGH, P., LANGRIDGE, P., WOLTERS, M.C., ALBERTSEN, A.M., CIGAN, R., WHITFORD (2017): Molecular identification of the wheat male fertility gene *Ms1* and its prospects for hybrid breeding. Nature Commun., *8*: 869.

UWE, M. (2018): Growth stages of mono- and dicotyledonous plants BBCH Monograph. Julius Kühn-Institut (JKI), Quedlinburg, 2018.

ZHANG, A., D., LIU, X., NIE, X., GUO (2001): Advances of Hybrid Wheat Breeding in China. Cereal Research Communications, 29(3-4): 343-350.

WANG, Z., J., LI, S., CHEN, Y., HENG, Z., CHEN, J., YANG, K., ZHOU, J., PEI, H., HE, X.W., DENG, L., MA (2017): *Poaceae*-specific *MS1* encodes a phospholipid-binding protein for male fertility in bread wheat. Proc. Natl. Acad. Sci. USA, *114*: 12614-12619.

WHITFORD, R., D., FLEURY, J.C., REIF, M., GARCIA, T., OKADA, V., KORZUN, P., LANGRIDGE (2013):Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. J. Exp. Bot., *18*: 5411-5428.

HEMIJSKI INDUKOVANA MUŠKA STERILNOST KOD OBIČNE PŠENICE POSREDSTVOM *AEGILOPS* CITOPLAZME

Primož TITAN[1,2], Jernej ISKRA[3], Koji MURAI[4], Vladimir MEGLIČ[5*]

[1] RGA, Raziskovalna genetika i agrokemija, Murska Sobota, Slovenija
[2] Univerzitet u Mariboru, Fakultet za poljoprivredne i biosistemske znanosti, Maribor, Slovenija
[3]Univerzitet u Ljubljani, Fakultet za kemiju i kemijsku tehnologiju, Ljubljana, Slovenija
[4]Univerzitet u prefekturi Fukui, Fukui, Japan
[5] Poljoprivredni institut, Odeljenje za oplemenjivanje i sjemenarstvo, Ljubljana, Slovenija

Izvod

U prošlosti, nekoliko načina je predloženo za razvoj hibridne pšenice. Međutim, u današnje vreme manje od jednog procenta svetske proizvodnje hlebne pšenice potiče od hibrida. Razlog za slabo širenje u proizvodnji hibridne pšenice leži u činjenici da nije pronađen jednostavan i efikasan sistem stvaranja hibridnog semena. Dvolinijski sistem koji koristi fotoperiod-senzitivnu citoplazmatičnu mušku sterilnost (FCMS) poreklom iz vrste *Aegilops crassa* Boiss. dosta obećava u pogledu stvaranja hibridnog semena pšenice u velikim razmerama. Međutim slabost FCMS sistema je zavisnost sterilnosti muških komponenti od geografske širine. Zbog toga smo pokušali da indukujemo citoplazmatičnu mušku sterilnost na bazi *Ae. crassa* hemijskim putem. Rezultati pokazuju da je većina gena muške sterilnosti konzervirana u citoplazmi *Ae. crassa* i da se njihovo delovanje može promovisati hemijskim stimulansom, kao što su sintetički auksini (npr. 2,4-dihlorofenoksi sirćetna kiselina). U radu je predstavljen razvoj novog sistema muške sterilnosti koji je kontrolisan hemijskim stimulansom (npr. sintetički auksini i njihovi analozi u formi pro-herbicida) i promovisan putem *Ae. crassa* citoplazme.

Primljeno 27.VII.2019.
Odobreno 18. XII 2019

.