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Doubled haploids of interspecific hybrids between *Brassica napus* and *Brassica rapa* for canola production with valuable breeding traits

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Abstract – Doubled haploids (DH) were obtained from two interspecific hybrids between *Brassica napus* and *Brassica rapa*. Seeds of doubled haploid plants differed in colour and size. The hybridity of the obtained doubled haploid is shown using genomic *in situ* hybridization (GISH) analysis. Evaluation of drought tolerance during seed germination on PEG-6000 showed the advantage of doubled haploid plants of interspecific hybrids over the parent cultivars. The oil from seeds of doubled haploid plants showed good nutritional value.

Keywords: *Brassica napus* / *Brassica rapa* / interspecific hybrid / doubled haploid / drought tolerance

Résumé – Haploïdes doublés d'hybrides interspécifiques entre *Brassica napus* et *Brassica rapa* pour la production de colza avec des caractéristiques de sélection avantageuses. Des haploïdes doublés (DH) ont été obtenus à partir d'hybrides interspécifiques entre *Brassica napus* et *Brassica rapa*. Les graines de ces plantes haploïdes doublés différaient en couleur et en taille. L'hybridité de l'haploïde doublé obtenu est démontrée par une analyse génomique par hybridation *in situ* (GISH). L'évaluation de la tolérance à la sécheresse pendant la germination des graines sur PEG-6000 a montré l'avantage des plantes doubles haploïdes issues d'hybrides interspécifiques par rapport aux cultivars parents. L'huile des graines de plantes doublement haploïdes a montré une bonne valeur nutritionnelle.

Mots clés : Brassica napus / Brassica rapa / hybride interspécifique / haploïde doublé / tolérance à la sécheresse

1 Introduction

The Climate of Kazakhstan is characterised by extreme volatility in meteorological conditions over the years and between seasons. At the same time, most of the farmland is located in high-risk environmental zones with low annual precipitation (150–320 mm) and droughts of varying intensity (Feher and Fieldsend, 2019). The main regional areas of spring rapeseed cultivation are located in the north of the country where crops are mostly rainfed (USDA-FAS, 2010). Therefore, the main factor affecting spring rapeseed yield is drought. Since spring rainfalls are the major source of water for plants in northern Kazakhstan, it is important to grow crop varieties with a shorter vegetative season (USDA-FAS, 2010). At the same time, rapeseed varieties cultivated for the food industry, known as "canola", need to have high-quality seed oil with a desired unsaturated fatty acid content (Kotlyarova *et al.*, 2007). Creating interspecific rape hybrids with a yellow shell as well as low levels of erucic acid and glucosinolates may help resolve some of the aforementioned problems.

The Brassicaceae family is unique in its capability of interspecific hybridization, and many methods for such hybridization have already been discovered. This in turn facilitates the creation of synthetic complexes from different species as well as from their polyploids. Thus, effective model systems can be created to study the influence of polyploidy on crop yield (Lukens *et al.*, 2006).

Interspecific hybridization of rapeseed can help to improve traits such as yield (Karim *et al.*, 2014), resistance to pests and diseases (Neik *et al.*, 2017) and the fatty acid composition of seed oil, increasing the contents of oleic and linoleic acids and reducing the erucic acid content (Iqbal *et al.*, 2006). In addition, it facilitates the production of seeds with a yellow

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coat that have a technological advantage for oil extraction, in contrast to dark-coloured seeds (Matthew *et al.*, 2009). While *Brassica napus* itself is an allopolyploid interspecific hybrids (Hu *et al.*, 2019), only a limited number of interspecific and intergeneric crosses in the Brassicaceae family have resulted in full-value hybrid plants (Kotlyarova *et al.*, 2007). Difficulties in creating interspecific hybrids are caused by incompatibility barriers that occur between different taxonomic groups at the time of pollination and occur all the way throughout the ontogenesis of hybrid plants (Karim *et al.*, 2014).

However, various combinations of interspecific hybrids can be quickly transferred into a homozygous state through an isolated microspore culture. This technology has been successfully introduced and widely implemented, particularly for the Brassicaceae family. It has successfully been applied for obtaining haploids and doubled haploids of species such as *B. napus* (Zhou *et al.*, 2002; Weber *et al.*, 2005), *Brassica oleracea* (Phippen, 1990), *Brassica carinata* (Barro *et al.*, 2001; Barro *et al.*, 2003), *Brassica juncea* (Lionneton *et al.*, 2001) and *Brassica rapa* (Gu *et al.*, 2003; Ferrie and Keller, 2005).

Haploid technology plays an important role in plant breeding. It allows to quickly identify the optimal gene combinations and reduce the time for creating new cultivars. Doubled haploids of more than 300 species have been reported (Wedzony *et al.*, 2009; Germana, 2011). Doubled haploids obtained *in vitro* can be applied not only in practical breeding, but also in genetic engineering and in cellular plant breeding. The advantage of producing doubled haploids in an isolated microspore culture is the rapid production of homozygous material, which can be immediately assessed for its suitability for use in breeding process. Thus, subsequent seed reproduction should not result in any deviation from quantitative and qualitative characteristics in the following generations (Snowdon *et al.*, 1997; Wen *et al.*, 2012). As a result of using this technology, we can get new genotypes that will allow breeders to quickly select the desired genotypes due to the homozygosity of doubled haploids.

The strategy for production hybrid can be greatly simplified by evaluating the offspring using cytological methods.

Genomic *in situ* hybridization (GISH) is a reliable method for identifying parental genetic material in hybrid genomes at the chromosomal level. An important advantage of GISH over other molecular-genetic methods for analysing hybrids is that GISH does not require prior knowledge of nucleotide composition of the genome and allows coverage of the entire genome (Snowdon *et al.*, 1997). It has therefore successfully been applied to identify parental chromosomes and to monitor the introgression of genetic material from donor to recipient in potatoes (Gavrilenko *et al.*, 2001), triticale (Aliyeva *et al.*, 2015), rapeseed (Snowdon *et al.*, 1997) and other cultures. The method is based on the visualisation of DNA sequences in chromosomes that differ between parents. Using labelled genomic DNA of one parent and unlabelled genomic DNA of the second parent, one can get a fluorescent signal that allows the differentiation between parental chromosomes in an interspecific hybrid by blocking homologous sequences (Marasek *et al.*, 2004). However, it is difficult to detect intergenomic rearrangements in *Brassica* chromosomes. Also, due to a concentration of heterochromatin around the centromeric regions, and the extensive intergenomic homoeology among the Brassicaceae, genomic probes often do not hybridize uniformly across the entire length of the chromosomes (Snowdon *et al.*, 1997).

A more precise determination of chromosomal rearrangements can be performed using the combined fluorescent/genomic *in situ* hybridization (FISH/GISH) method (Abbasi *et al.*, 1999; Cao *et al.*, 2000). It can be applied in hybridization with the whole genomic DNA as a probe to distinguish genomes as well as with chromosome-specific DNA probes to either identify mitotic chromosome pairs or to visualise the pairing of homologous chromosomes in meiosis (Maluszynska and Hasterok, 2005; Weerakoon, 2011). In addition, the GISH method allows the chromosomes of genomes A and B in *B. juncea* to be distinctly labelled (Weerakoon *et al.*, 2009). Multicolour FISH with an rDNA probe in combination with GISH helped to identify 28 chromosomes of these species (Maluszynska and Hasterok, 2005).

One of the limiting stress factors for cultivating canola is drought. This environmental condition significantly affects yield at the flowering stage (Xie *et al.*, 2013) and seriously influences the fatty acid composition of seeds, particularly oleic and erucic acid contents (Yuan *et al.*, 2015).

In comparison to *B. napus*, *B. rapa* may harbour a superior and more rapid response to drought stress (Guo *et al.*, 2017). Accordingly, hybrids between these species may be more drought-tolerant than rapeseed *B. napus*. For determine tolerance to drought, experiments are carried out method of simulating drought using a hypertonic solution of polyethylene glycol (PEG-6000). Using of PEG is one of the appropriate procedures to create drought conditions for screening some parameters of drought tolerance, such as germination, plant height, root length, relative water content (RWC) (Basha *et al.*, 2015). Some authors confirm that simulating drought using PEG in plants consists in influencing the lipid composition of membranes, which is a key component in photosynthesis (Wang *et al.*, 2020), and also leads to some changes in the structure of chloroplasts (Khan *et al.*, 2019). Using PEG-6000 to simulate drought is a very simple and fast method for early detection of drought tolerance (Xie *et al.*, 2013).

Therefore, the main aim of this work is to produce doubled haploids of hybrids between *B. napus* and *B. rapa*. The resulting doubled haploids (a) determine the degree of their hybridity using GISH analysis; (b) determine their prospects for resistance to drought using PEG-6000; (c) determine their fatty acid composition according to the technical parameters necessary for canola.

2 Materials and methods

We used previously obtained (Zhambakin *et al.*, 2017) hybrid seeds of two combinations: Kris (*B. napus*) × Zolotistaya (*B. rapa*) (BKZ) and Kris (*B. napus*) × Yantarnaya (*B.rapa*) (BKY). To obtain hybrid seeds, we used one cultivar of rapeseed *B. napus* (Kris – All-Russia Research Institute of Agricultural Biotechnology, Moscow, Russia) and two cultivars of rape (*B. rapa* ("Zolotistaya" and L. "Yantarnaya") – Federal state budgetary scientific institution "Federal scientific center" "V.S. Pustovoit All-Russian Research Institute of Oil crops").

2.1 Isolation of a microspore culture

Buds (2–3 mm in size) were collected in field conditions early in the morning at the single-nucleus microspore stage during hours of intense pollen division. The pretreatment of buds was carried out in a 10 mg/l silver nitrate solution at +4 °C for 2 days. Subsequently, the buds were sterilised with 5% sodium hypochlorite for 7–10 min and 70% alcohol for 3–5 s, followed by three washes with distilled water. The buds were then placed in a cool micromixer (10 °C) using 30–40 ml of cooled B5 medium (Gamborg and Eveleigh, 1968) without hormones (10–12 °C) and homogenised for 7–9 s at high speed. The resulting suspension was passed through a filter (80 μm), and the filtrate was centrifuged (Eppendorf, Germany) at 100 g for 5 min. The supernatant was decanted, and 15 ml of the B5 medium were added to the precipitate, followed by centrifugation for 5 min. After repeating the previous step, the precipitate was poured into Petri dishes. Subsequently, NLN medium with 0.05 mg/l benzylaminopurine (BA) was added for microspore cultivation. The concentration of microspores in the NLN medium was adjusted to 35 000–50 000 microspores per ml. Petri dishes were placed in a temperature controller with a shaker at 25 °C. As soon as torpedo-like embryoids appeared, the Petri dishes were exposed to light at the same temperature (Swanson, 1990).

Embryoids derived from the isolated microspore culture were transplanted onto Gamborg B5 solid nutrient medium supplemented with 1 mg/l gibberellic acid. As regeneration proceeded, the plants were transplanted onto Murashige-Skoog hormone-free medium with half salt composition, one plant per tube, and placed into a room with controlled light and temperature.

2.2 Colchicine

Regenerated plants were cloned *in vitro*. The clones were then germinated to the stage of five leaves and treated with 0.05% colchicine. After that, treated plants were washed three times with distilled water, transferred to soil and grown until the formation of seeds in doubled haploid plants of interspecific hybrid combinations (Gland, 1981).

2.3 GISH analysis: preparation of microscope slides

To obtain mitotic metaphase plates, the seeds of hybrids were germinated on moist filter paper in Petri dishes for 2 days. Subsequently 1.5–2-cm long roots were pretreated in 2 mM 8-ortho-oxyquinoline for 4 h at 25 °C and fixed in an ethanol + acetic acid solution at a ratio of 3:1. Fixed roots were washed in citrate buffer (0.01 M citric acid + 0.01 M sodium citrate, pH 4.8) and treated with an enzymatic mixture of 1% cellulase (Sigma Aldrich, USA) and 2% pectinase (Sigma Aldrich, USA) for 1.5–2 h at 37 °C. After separation from the non-meristematic parts, root tips were squashed in a drop of 45% acetic acid and the preparations were frozen (Hasterok *et al.*, 2005).

2.4 DNA labelling and GISH

DNA labelling and GISH were performed according to a method described previously (Hasterok *et al.*, 2005). The DNA of seed parents was labelled with digoxigenin-11-dUTP (Roche, Mannheim, Germany) (*B. rapa*) and rhodamine-dUTP (Roche, Mannheim, Germany) (*B. napus*) fluorochrome labels using the nick-translation (Sigma Aldrich, St. Louis, MI, USA) method. The samples were pretreated with RNase (without DNase, 100 μg/ml, 1 h at 37 °C) and washed three times in 2 × SSC buffer (saline-sodium citrate ($NaCl + Na_3\text{-}C_6H_5O_7 \times 5.5H_2O + H_2O$). Post-fixation was performed in a 4% aqueous formaldehyde solution. The hybridization mixture consisted of 50% deionized formamide, 10% dextran sulphate, 2 × SSC, 0.5% SDS and the DNA probe. The hybridization mixture was preliminarily denatured at 75 °C for 10 min and then applied to the slides. The samples and the hybridization mixture were denatured together at 75 °C for 5 min and left to hybridize overnight in a humid chamber at 37 °C. After stringent washing (20% formamide in 0.1 × SSC at 42 °C, 2 × 5), immunodetection was performed with FITC-conjugated anti-digoxigenin (Roche, Mannheim, Germany). Final samples contained 2 μg/ml DAPI in Vectashield antifade mounting medium (Vector Laboratories, Burlingame, CA, USA).

2.5 Determining the drought tolerance using polyethylene glycol 6000 (PEG-6000).

Hybrid seeds were presoaked in water for 24 h. Subsequently, the seeds were sterilised in sodium hypochlorite for 10 min, then in 70% ethanol for 3–5 s, and finally thoroughly washed with sterile water three to four times. After sterilisation, the seeds were sown in Petri dishes (10 pieces per dish) with PEG-6000-impregnated filter paper and incubated at 25 °C in a 16 h/8 h day/night photoperiod. Observations were carried out daily. Hypocotyl and root growth indicators were recorded over 8 days (Xie *et al.*, 2013).

2.6 Evaluation of the fatty acid composition

Samples for chromatography were prepared as follows: 0.5 ml of seed oil (10 seeds that were produced under the same environmental conditions) was extracted in a mill. Then, 8 μl of the oil were collected with a pipette into a glass tube. There after that, 2 ml of hexane and 0.1 ml of 5% sodium methylate were added to the oil. The tube was left to sit for 30 min with periodical shaking at least three times. After the incubation period 1 ml of distilled water was added to the tube, and the tube was then shaken and allowed to settle. Subsequently, 1 ml of the upper hexane layer was transferred to a penicillin vial, which was placed under a fan at room temperature until the hexane was completely evaporated. After drying, 600 μl of chemically pure hexane were added to the vial. The fatty acid composition was determined by gas chromatography (GOST R, 1999).

2.7 Statistical analysis

Significant results were tested using Analysis of Variance (ANOVA) by applying the Fisher LSD test with with the program SPSS 22 (IBM). Means with different letters are regarded as statistically significant at $p < 0.05$.

3 Results

3.1 Obtaining doubled haploid seeds of interspecific hybrids

The previously obtained seeds (F1) of hybrid combinations BKZ and BKY were sown on the experimental field. Cultivated hybrid plants (F1) were used to produce doubled haploids of interspecific hybrids by isolated microspore culture.

Figure 1 illustrates the developmental stages from an isolated microspore through numerous divisions to the production of a bipolar embryo and its derived regenerated plants. In total, 152 to 162 embryos per 100 buds were derived using an isolated microspore culture from two hybrid combinations: BKZ, BKY. Depending on the hybrid combination, the percentage of regenerants derived from embryos varied between 38.8 and 43% (Tab. 1).

In this experiment, only part of the embryos from each combination was used to obtain haploid plants, and only some of them were treated with colchicine. Another part of them was cloned for other experiments. Most colchicine-treated plants were sterile (Tab. 1).

The resulting doubled haploids of interspecific hybrids seeds differed phenotypically from the parents *B. napus* and *B. rapa* cultivars. The DHBKY hybrid combination produced yellow-brown seeds, while the seeds of the DHBKZ hybrid were brown (Fig. 2). The seeds of the DHBKZ combination hybrid were small, similar to *B. napus* seeds. At the same time, the weight of 1000 seeds of cultivar Kris was 3 g, and in doubled haploids of interspecific hybrids plants, it varied from 1.1 to 4 g.

Thus, seeds of doubled haploids of interspecific hybrids were obtained, which differed in colour, size, as well as in quantitative traits, the weight of 1000 seeds. Further, the obtained hybrid seeds of doubled haploids were used to (a) determine the hybridity using GISH analysis; to (b) assess resistance to drought in laboratory conditions; to (c) quantifity their fatty acid composition.

3.2 GISH analysis

In our case, we wanted to show the hybridity of doubled haploids obtained in the culture of isolated microspores *B. napus* × *B. rapa*. Figure 3 shows a GISH analysis of the obtained seeds of doubled haploids of interspecific hybrids of DHBKZ, which were stained with DAPI and contained chromosomes from the genome of the donor plant. Fluorescent signals are clearly present on each chromosome of the hybrid. The predominant localization of hybridization signals was observed in the centromeric region. In seven doubled haploids of interspecific hybrids chromosomes, hybridization signals were much stronger in the centromere regions of the chromosomes, while the telomere signals were weak. The GISH analysis clearly showed the presence of *B. rapa* DNA in seven chromosomes of *B. napus* × *B. rapa* hybrid combination. It is likely that the yellow colour of the seeds was transmitted to *B. napus* from the chromosomes of *B. rapa* genome A.

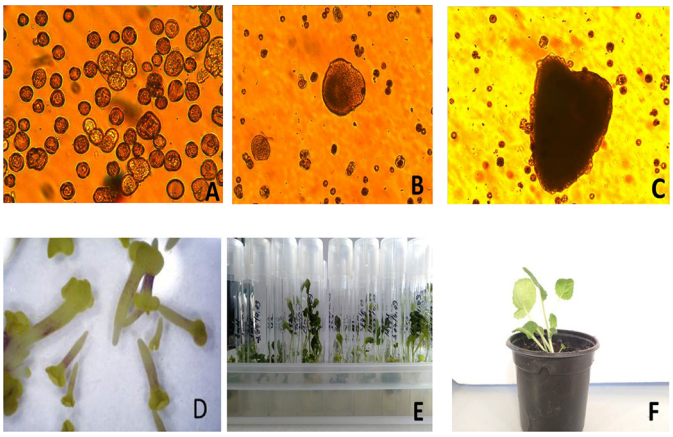

Fig. 1. Embryogenesis of microspores of interspecies hybrids of *B. napus* × *B. rapa.* (A) Microspores that had been cultivated for one week. (B, C) Globular- and torpedo-shaped embryos. (D) Microspore-derived embryos (MDE). (E, F) Regenerated plantlets from embryos. Bar = 100 μm.

3.3 Drought tolerance test

Figure 4 shows the results of drought-tolerance tests of doubled haploids of interspecific hybrids and their parents. The doubled haploids of interspecific hybrids differed from the parents in the percentage of seed germination and relative water content in seedlings. Seed germination of all varieties of parents and doubled haploid of interspecific hybrids in the control group (0% PEG) began after 24 h and reached 100%. With the increase in the concentration of PEG-6000, the rate of seed germination of *B. napus* significantly decreased.

The seed germination rate of *B. napus* cultivars (female parents) at 20% of PEG-6000 made up only 33%, while *B. rapa* cultivars showed much higher rates: "Yantarnaya" *B. rapa* obtained 66%, while "Zolotistaya" reached 93%. Compared with the parent seeds, seeds of doubled haploids of interspecific hybrids showed average results around 60%.

The relative water content in *B. rapa* seedlings was higher than that in *B. napus*. The highest percentage of water content was registered for seedlings of *B. rapa* L. "Yantarnaya", which made up 62%, followed by *B. rapa* L. "Zolotistaya" (56.5%) at 20% of PEG-6000. The water content in seedlings of the *B. napus* cultivar ("Kris") was about 46%.

The amount of water in seedlings of doubled haploids of interspecific hybrids was higher than that in parental seedlings. The relative water content in the seedlings was 75.4% for hybrids of DHBKZ and 70% for hybrids of DHBKY.

3.4 Fatty acid composition of doubled haploid seeds of interspecific hybrids

Table 2 presents the results of doubled haploids of interspecific hybrid fatty acid composition analysis. Erucic acid was not detected in any seed. In terms of oleic acid content, the DHBKY doubled haploid of interspecific hybrids had the highest percentage of oleic acid (68.94%). At the same time, the amount of saturated fatty acids of interspecific hybrids remained relatively low compared to *B. napus* and *B. rapa*.

Table 1. Embryogenesis, regeneration, and seed production of doubled haploids of F1 interspecific hybrids of *B. napus* × *B. rapa.*

Name of hybrid combination	Number of formed embryos/100 buds	Number of plants from embryos		Number of plants treated with colchicine	Number of fertile plants from embryos *n*/%
		n	%		
F1 BKZ	152	161	42.3	72	31/43
F1 BKY	162	139	31	72	28/38.8

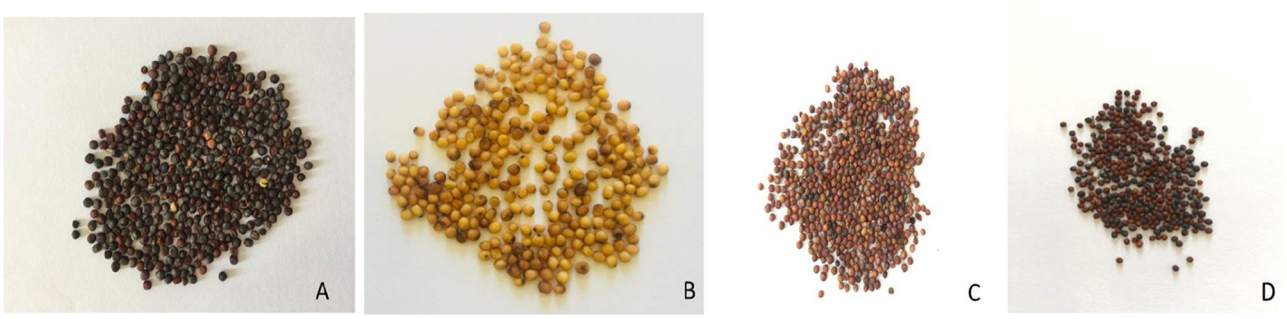

Fig. 2. Seeds of doubled haploids of interspecific hybrids *B. napus* × *B. rapa*. (A) Seeds of *B. napus*. (B) Seeds of *B. rapa*. (C) Yellow-brown seeds of the hybrid DHBKY. (D) Small brown seeds of the hybrid DHBKZ.

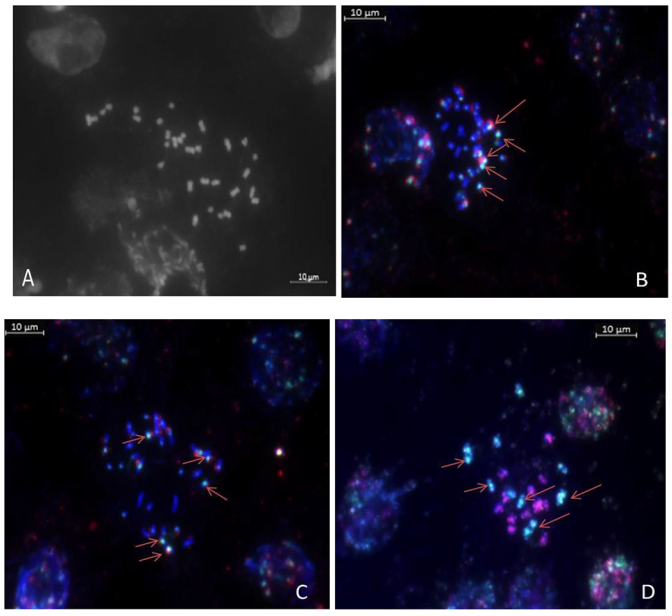

Fig. 3. Genomic *in situ* chromosome hybridization of DHBKZ. In all images, chromosomes are stained with DAPI (blue) and probed for the genomic DNA of *B. napus*, fluorescently labelled with rhodamine-5-dUTP (red), and the genomic DNA of *B. rapa* labelled with digoxigenin-11-dUTP (green). (A) Chromosomes of hybrids under light microscope, 2n-33 chromosomes. (B, C, D) GISH analysis of interspecific hybrid of *B. napus* x *B. rapa* (arrows indicate chromosomes transmitted from *B. napus*).

4 Discussion

Interspecific hybrids of the Brassicaceae family are excellent material for producing doubled haploids using an isolated microspore culture (Zhou and Scarth, 1995). As a result due to the allopolyploid nature of *B.napus*, a completely new homozygous and, at the same time, hybrid plant may appear, having the most diverse combination of chromosomes of *Brassica* species (Li *et al.*, 2018). In our experiment, we obtained a rather high embryogenesis productivity in a culture of isolated microspores and a rather high level of plant regeneration. As a result of colchicine treatment, more than a third of the plants were fertile. Among the obtained hybrids, plants with an intermediate seed colour from dark yellow to brown were noted.

In the present work, GISH analysis was used to confirm the hybrid nature of doubled haploids of interspecific hybrids of *B. napus* and *B. rapa*. This method has been successfully applied to identify parental chromosomes in hybrids with small chromosomes in *B. napus* breeding (Hasterok *et al.*, 2005). The GISH method, performed on doubled haploids of interspecific hybrids of *B. napus* and *B. rapa*, allowed us to clearly identify the chromosomes of the parental species in the nucleus of doubled haploids of interspecific hybrids. In the metaphase of doubled haploids of interspecific hybrids, GISH analysis showed the origin of conjugation of chromosome regions. But our results using GISH hardly resemble the typical "chromosome painting" image of hybridization with the total genomic DNA probe. Despite this, as shown in Figure 2, the GISH method was effectively applied for the detection of parental genomes in *Brassica* hybrids. A lack of moisture is a major deterrent to the cultivation of rapeseed in Kazakhstan. Therefore, selection for the cultivation of drought-tolerant varieties and hybrids of rapeseed (canola) is an urgent problem. Channaoui *et al.* (2019) carried out work on the assessment of drought resistance and the use of PEG-6000 of the Brassica family. Similarly, researchers from China (Yang *et al.*, 2007) tested for drought-resistance using PEG-6000 on 14 varieties of rapeseed.

In our experiment, we indirectly evaluted the resistance to drought of plants of the doubled haploids of interspecific

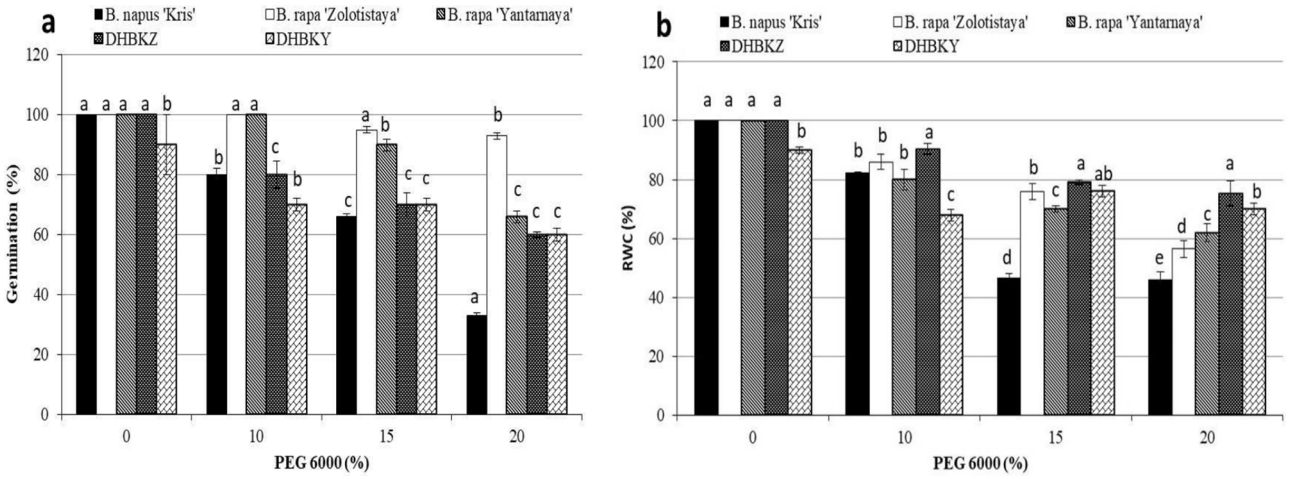

Fig. 4. Effect of PEG-6000 induced water stress on seed Germination (a) and on Relative Water Content (RWC); (b) of controls (*B. napus*, *B. rapa*) and hybrids seedlings (DHBKZ, DHBKY). Data are means ± SD bars of three experiments ($n = 20$ seeds per experiment), measured in triplicate. The same letter indicates no significant difference and the different letters indicate a significant difference (Fisher's LSD, $P < 0.05$).

Table 2. Fatty acid composition of the seed oil of the parents and doubled haploids of interspecific *B. napus* hybrids.

Name		Fatty acid concentration, %						
		P (C16:0)	S (C18:0)	O (C18:1)	L (C18:2)	Ln (C18:3)	B (C22:0)	Er (C22:1)
Parents (Control)	*B. napus* "Kris"	4.5	2.1	61.9	19.5	6.2	0.4	< 0.05
	B. rapa "Zolotistaya"	5.7	1.6	51.7	28.1	7.3	0.1	< 0.05
	B. rapa "Yantarnaya"	4.7	1.8	53.8	24.9	6.8	0.2	< 0.05
Doubled haploid hybrids	DHBKZ	3.6	2.1	67.8	18.1	5.7	0.2	< 0.05
	DHBKY	3.9	2.9	68.9	18.3	2.2	0.2	< 0.05

P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; Er: erucic acid; B: behenic acid.

hybrids in comparison with plants of *B. napus* and *B. rapa* by the percentage of seeds germinated in an osmotic solution of various concentrations. The physiological basis of this method is the ability of plant seeds to germinate in PEG-6000 solution. A high percentage of sprouted seeds reflects the ability of the genotype to use scarce soil moisture reserves, which indicates its resistance to drought in the initial stages of development. Assessment of drought resistance during seed germination on PEG-6000 in our experiment showed the advantage of a doubled haploid of interspecific hybrids over the varieties of parents *B. napus* and *B. rapa*.

Rapeseed is grown in Kazakhstan for the production of edible oil (canola) both for domestic consumption and for export. The quality of canola oil is determined mainly by the high contents of unsaturated (oleic, linoleic, linolenic) and low saturated (palmitic and stearic) fatty acids; in addition, the content of erucic acid should not exceed 2%. At the same time, the requirements for oil quality are increasing constantly. In particular, vegetable oils with a high content of oleic acid are relevant for a healthy diet (Bowen *et al.*, 2019). The fatty acid composition of the doubled haploid seeds obtained by us fully meets the modern requirements for canola cultivars.

In our work, the obtained doubled haploid plants were characterised by resistance to arid growing conditions, and the seed quality met the technological requirements for canola. Further work should aim at testing the doubled haploids of interspecific hybrids in the field conditions of northern Kazakhstan to create new canola cultivars (000).

Abbreviations

BA	Benzylaminopurin
GISH	Genomic *in situ* hybridization
PEG	Polyethylene glycol

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