Development of homozygous lines of open-pollinated and self-incompatible plants *Brassica hirta L.*

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White mustard syn. yellow mustard (*Sinapis alba* L. syn. *Brassica hirta*) is open-pollinated, an annual plant of the family Brassicaceae. *Sinapis alba* as an oil plant is of growing economic importance due to the multilateral uses for example: seeds for food production, as green manure crops, phytosanitary crops and valuable honey plant and other. White mustard among Brassicaceae is the most resistant to drought occurring in Poland late spring and in summer. The seeds of traditional cultivars of white mustard, including cv. **Nakielska**, is characterized by high erucic acid content in oil (35-37%) and high content of glucosinolates, especially sinalbine (approx. 160 µmol/g-1), which remain in the extracted meal. In contrast, newly developed cv. **Bamberka**, has erucic acid content maximum 1.3 %, but content of glucosinolates similar to traditional cultivars. The first in Poland, double improved white mustard cv. **Warta** contains less than 1.5 % of erucic acid in oil and less than 20 µmol g-^{1} of glucosinolates in meal, sinalbine is complitely eliminated (Table 1).

Tab. 1. Characteristics of three varieties of white mustard (*Sinapis alba*). Results from field experiments conducted in two stations

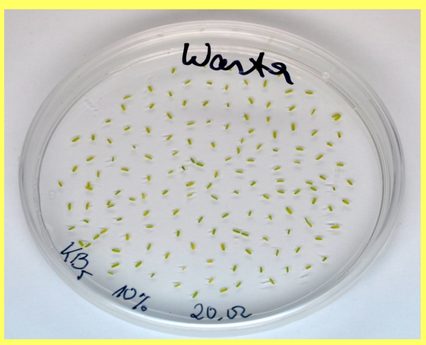
Cultivar	Traits					
	Seed yield (dt ha^{-1})	1000 seeds wight (g)	Oil content (% dry wight)	Erucic acid content (%)	Glucosinolates (µM g^{-1})	Sinalbin (µM g^{-1})
Nakielska	22.4	7.2	27.8	36.6	161.7	156.2
Bamberka	17.3	5.7	29.6	1.3	136.7	132.5
Warta	20.2	6.3	30.2	1.3	20.4	0.0

Piętka et al. 2014

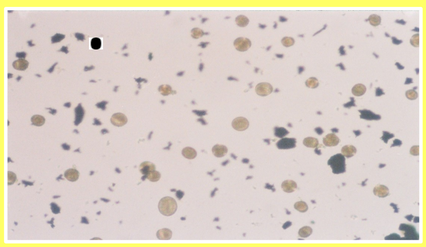
The objectives of the study were to develop a microspore culture protocol for three different cultivars *S. alba*: high erucic acid and high glucosinolate content - Nakielska, low erucic acid and with high glucosinolate content - Bamberka and double improved - Warta.

While in our previous studies observed a negative impact on *Sinapis alba* microspores rich in mineral and organic compounds NLN medium (Lichter, 1982) we have used KB5+B5 medium (Leelavathi et.al 1984). Microspores of white mustard were isolated from anthers which were cultured on solid medium KB5+B5 after exposure to low temperatures 4°C. The first divisions of microspores were observed after a few days of isolation from anthers. Over the next two weeks were recorded multicellular divisions and then forming the embryos. Visible to the naked eyes embryos were transferred to fresh KB5+B5 liquid medium and cultured at light. Green embryos were planted on stable MS medium. After a few weeks, were observed the formation of shoot primordia. Developed shoots on MS medium with kinetin were transferred to rooting MS medium with IBA (Klóska et al. 2012). Androgenic plants of white mustard were planted to the soil and their further development took place in greenhouse until the seed were collected.

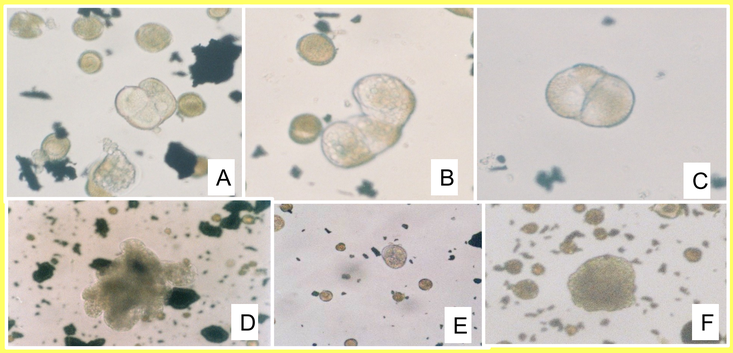
***Sinapis alba* L.**



Anther culture



Microspore culture in KB5+B5 medium



Divisions of microspores after a few days in culture (cv. Nakielska – A, B, C; cv. Warta – D, E; cv. Bamberka – F)

White mustard embryos on KB5+B5 medium

Shoot organogenesis on MS medium with kinetin

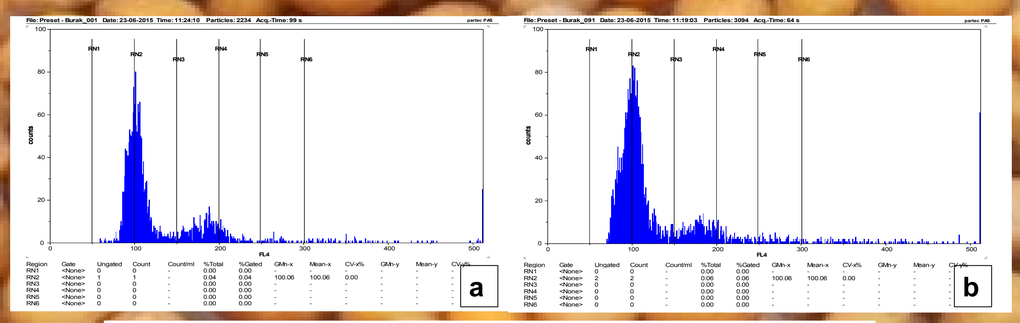
Regenerated plantlets on rooting medium (MS with IBA)

Regenerated androgenic plant in the soil

Androgenic plant of *Sinapis alba* obtained from cv. Warta

Tab. 2. Efficiency of androgenesis *in vitro* in isolated microspores culture of white mustard (*Sinapis alba* L.)

Cultivar	No of embryos per anther			
	I serie	II serie	III serie	average
Nakielska	0.10	0.33	0.41	0.28
Bamberka	0.20	1.0	0.80	0.67
Warta	0.50	0.2	0.32	0.32



Flow cytometric analysis for *Sinapis alba*:
a/ androgenic plant obtained from cv. Warta b/ cv. Warta

Conclusion

Doubled haploid lines of *Sinapis alba:* traditional, low erucic acid and double improved cultivars were developed by microspore culture.

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This work was supported by the Ministry of Agriculture and Rural Development, Poland Project HOR hn No 52

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