Andini, R. · M.I. Sulaiman · M. Muzaifa · Y.D. Fazlina · C. Möllers

Oil bodies sizes variation analyses of rapeseed in two locations as a novel trait for genetic engineering

Abstract. Rapeseed (*Brassica napus* L.*)* containing oil content from 33 up to 48% (on 8.5% moisture basis) is the major source of oil plant in many temperate regions, *e.g.* in Germany. It is mainly applied for cooking, bio-diesels; and animal fodder. Seed plants (soybeen, rapeseed, sunflower) store oil in a storage organelle called oil body whose size varies from 0.6 – 2.0 µm, depending on the plant species. Increasing the oil content is one of the breeding targets in many of oil plants, including in rapeseed. Due to increasing awareness of the environment and the hazardous impact of solvent extraction agents; such as *n*-hexane (C_6H_{14}) on human health, their application in the oil extraction process is slowly being reduced. A more friendly oil extraction method *via* centrifugation was introduced over the past decade as well as for biotechnological application. Each 200 mg of *B. napus* L. *cv*. 'Maplus' seeds were applied as material in this study. Seeds originated from the Double Haploid (DH) population grown in two significantly distinct environments in China and Germany. The average of oil content from two populations was also different, namely 49,18% in China, and 56,94% in Germany. In this study, oil bodies were isolated *via* the centrifugation method and their distribution was observed under the light microscope. Based on the Coulter Counter measurement, the diameter sizes were ranging from 1,03 - 1,07 µm (*mean*= 1,05 µm) and 0,98 - 1,02 µm (*mean*= 1,00 µm) in German and Chinese genotypes, respectively. This study confirms a positive and very highly significant correlation between the size of oil bodies and oil content in rapeseed.

Keywords: Centrifuge · Coulter counter · Deutschland` · Oil plant

Analisis variasi ukuran 'oil body' pada tanaman raps di dua lokasi sebagai karakter baru untuk perakitan tanaman

Sari Tanaman raps (*Brassica napus* L.) adalah tanaman penghasil minyak utama di negara dengan iklim dingin seperti di Jerman. Minyak raps (*Brassica napus* L.*)* digunakan untuk bahan pangan, biodiesel, dan sebagai pakan ternak. Kandungan minyaknya bisa mencapai 33 – 48%. Tanaman minyak seperti kedelai (*Glycine max*), raps (*Brassica napus* L.), bunga matahari (*Helianthus annuus*) pada umumnya menyimpan kandungan minyaknya dalam suatu organela penyimpanan dikenal dengan 'oil body' yang mempunyai diameter antara 0,6 hingga 2,0 µm, tergantung dari spesies tanaman. Peningkatan kandungan minyak merupakan salah satu target pemuliaan di banyak tanaman minyak, termasuk di raps. Tulisan ini menceritakan tentang isolasi 'oil body' dari tanaman raps menggunakan metode sentrifugasi, yang dapat mengurangi efek negatif dari *n*-heksan sebagai zat ekstraktor yang lazim digunakan dalam proses penyulingan minyak. Sebanyak 200 mg benih *B. napus* L. kultivar 'Maplus' digunakan sebagai bahan dalam penelitian ini. Benih berasal dari populasi Double Haploid (DH) yang ditanam di dua lingkungan yang berbeda secara signifikan di Cina dan Jerman. Rata-rata kandungan minyak dari dua populasi juga berbeda, yaitu 49,18% di Cina, dan 56,94% di Jerman. Dalam penelitian ini, 'oil body' diisolasi melalui metode sentrifugasi dan distribusinya diamati di bawah mikroskop cahaya. Berdasarkan pengukuran partikel 'Coulter Counter', diameter 'oil body' pada tanaman raps bervariasi antara 1,03 - 1,07 µm (rata-rata= 1,05 µm) pada genotipe dari Jerman, dan 0,98 - 1,02 µm (rata-rata= 1,00 µm) pada genotipe Cina. Selain itu, studi ini mengkonfirmasi korelasi positif dan sangat signifikan antara ukuran 'oil body' dengan kandungan minyak di tanaman raps.

Kata kunci: Coulter counter · Deutschland · Sentrifugasi · Tanaman minyak

Diterima : 10 Maret 2021, Disetujui : 1 Agustus 2021, Dipublikasikan : 1 Agustus 2021
DOI: https://doi.org/10.24198/kultivasi.v20i2.32595

Andini, R.[1] · M.I. Sulaiman[1] · M. Muzaifa[1] · Y.D. Fazlina[1] · C. Möllers[2]
[1] Fakultas Pertanian, Universitas Syiah Kuala (USK), Tgk. Hasan Krueng Kalee No. 3, Banda Aceh, Indonesia
[2] Department of Crop Sciences, Von-Siebold Str. 8, D-37075, Göttingen, Germany
Korespondensi: rita.andini@unsyiah.ac.id

Jurnal Kultivasi Vol. 20 (2) Agustus 2021
ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Rapeseed *(Brassica napus* L.*)* or also known as 'canola' is the major source of oil plant in many temperate regions, *e.g*. in Germany with the oil content is ranging from 33 - 48% (8.5% moisture basis). It is mainly used for cooking, in industrial uses such as for bio-diesels; and as animal fodder (Spasibionek *et al*., 2020). The total planted area is estimated approximately 1.5 million hectares (Strohm, 2010).

Genetic engineering is a new art in plant biotechnology in order to create 'new' plants with novel traits *via* the manipulation of plant genomes**.** With this new method**,** many agronomic traits could be manipulated that are in accordance with the breeder's intention and such transgenic plants could be incorporated in a larger role in future plant improvement programs in food & nutritional security, feedstocks, and nutraceutical purposes. Many oil plants *e.g*. maize, soybean, and canola or rapeseed have been manipulated; with the major breeding intention is to increase their content of oil (Kumar and Thompson, 2010)

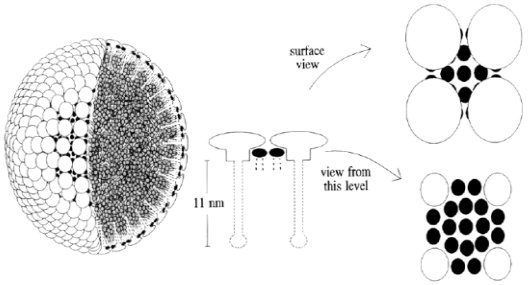

Figure 1. The structure of oil body consisting of Triacylglycerols (TAG)-matrix and surrounded by the phospholipid layer; embedded with oleosins (depicted and modified from Huang, 1996).

Seed plants store oils in a storage organelle or in specialized tissues during seed development termed as oil body, whose spherical sizes are varied from 0.5 to 2.0 µm, depending on the plant species (Figure 1). The oils are majorly stored in the embryo, while, the amount of storage oils are approximately 4% (w/w). As a matter of fact, diverse oil crop plants, *e.g.* soybean (*Glycine max*), rapeseed (*Brassica napus*), and sunflower (*Helianthus annuus*), and particularly the so called "*Illinois High Oils"* (IHO); one of maize (*Zea mays*) varieties containing high oil content were to be reported to have diverse seed oil contents in the seeds (Ting *et al.,* 1996). Thus, a significant different ratio of oils *vs*. oleosins in their seeds was exist; depending on the synthesis of oils'- and oleosins' gene expression or oil-body *biogenesis* during seed maturation at a later stage (Millichip *et al.,* 1996).

Cunxu *et al.* (2009) reported that there is a tendency smaller oil bodies have a spheroid form, meanwhile the larger ones are ovoid. Furthermore, two structural proteins: i) oleosin; ii) caleosin are currently associated with the structure and stabilization of oil bodies (Frandsen *et al*., 2001). Oleosin might have been derived from caleosin based on a putative evidence of gene expression responsible for encoding caleosin; after a long term of divergent evolution. Nevertheless, both play a great role in maintaining the integrity of these lipid storage organelles and oil-body *biogenesis* (Jiang and Tzen, 2010).

Oleosins appear to be structural proteins with a molecular weight ranging from 15,000 to 26,000; depending on the species. Their existence has received considerable attention in the past decade, due to their essential role in the oil-body genesis and their structural role in stabilizing the triacylglycerols (TAG)/ cytosol oil-body interface at the surface layer (Ting *et al*., 1996).

The breeding targets in most of oil plants are usually: (i) increasing the oil content, (ii) modifying the fatty acid components, (iii) enhancing one or few characeristiscs in tems of their nutritional components. Related to point (i), the so called 'Double-Haploid' (DH) breeding method is applied in various commercial crops, *e.g*. in maize , wheat, barley, and tobacco. A Double Haploid (DH) refers to a process of a genotype product, whose haploid cell (*n*) has already contained one copy of chromosomes that are originated from one of the parents, while the copies from the other parent are gone. Afterwards, the haploid cell undergoes chromosome doubling usually *via* an addition of chemical substance, likewise *colchicine* , so that the number of chromosome of the novel organism would be 2*n* (Figure 2). Haploids become valuable when scientists double the number of their chromosome in order to produce homozygous breeding lines.

In homozygous lines, furthermore, all genes on each pair of chromosomes in every cell of the plant are 'identical'. These homozygous lines are 100-percent inbred lines, which otherwise would have to be produced by

repeated 'forced' and natural self-pollinations. However, this may take some time. Therefore, Double Haploid could speed up a breeding process in a commercially valuable lines (Ren *et al*., 2017).

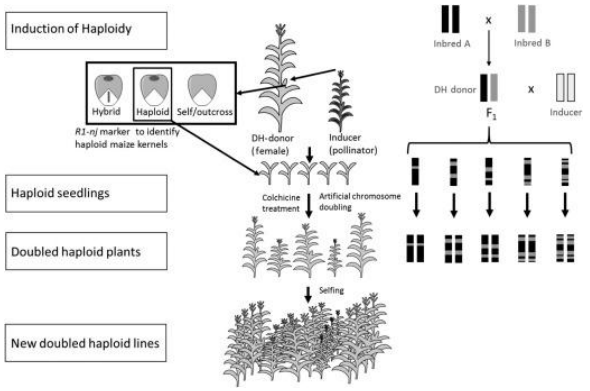

Figure 2. As an example: the process of Double Haploid lines production in maize (*Zea mays*) (Khan and Croser, 2004)

In terms of processing, the application of solvent extraction agent *e.g. n*-hexane (C_6H_{14}) is majorly applied by the oil extraction process, due to its good property as an extraction agent and cheap price. However, with an increasing awareness towards sustainable environment and the deteriorated impact to human health, the application of solvent extraction is slowly being reduced. Therefore, a more friendly oil extraction method via centrifugation was introduced in 2001. The objective of such process is to yield as much as possible the whole oil bodies; both the big and small ones through the centrifugal force and placed them at the surface of the liquid.

Wäsche (2001) designed some essential parameters; *e.g.* high centrifugation speed, cold condition being applied throughout the process in order to retain valuable and sensitive constituents, *e.g.* vitamins after the process, which is highly valued in biotechnological application such as in the '*Friolex*' oil extraction process. This recently developed method could amount the yield of oil up to 72%. This amount was still deliberately lower compared with the conventional method that could yield oil up to 85%. Despite a high centrifugation speed,it seemed that the very tiny oil bodies still remain at the bottom of liquid, thus, reducing the yield of oil bodies.

There is a positive correlation between the oil content in maize and the size of oil bodies, and in this study, we would like to investigate, whether this can be also extended to rapeseed. Therefore, the objectives of this study are: (i) to establish a process of isolation of oil bodies in rapeseed (*B. napus*) *via* sucrose gradient centrifugation; (ii) to analyze the size variation of oil body's diameter from a diverse set of genotypes in rapeseed with different oil content *via* Coulter Counter; (iii) to determine a correlation between oil content and oil body diameter in rapeseed.

Materials and Methodology

Materials: Each 200 mg seeds of *B. napus* L. cv. Maplus originated from 284 genotypes derived from Double-Haploid (DH) population grown in two locations: (i) China; (ii) Germany.

Methodology: all laboratory work was conducted at the Department of Crop Sciences (former: Plant Agronomy and Breeding Institute, at the 2nd floor, Von-Sieboldt Strasse in Göttingen, Germany).

I) *Oil body isolation*: two steps were conducted (I.1.; I.2.) prior to the isolation of oil bodies according to Ting *et al*. (1996).

I.1) *Preparation of crude extract and oil bodies*. 200 mg of rapeseed and 3-ml grinding medium (0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$, 2 mM dithiothreitol, 0.15 M Tricine-KOH, pH= 7.5) were placed in a centrifuge tube and then, homogenized with an ultra turrax (Euroturrax) at 27,000 rotation per minute (*rpm*) at 4 ^oC for 40 seconds (*sec.*). All chemicals applied were obtained from Sigma Aldrich, Germany.

I. 2) *Centrifugation*: a two instead of three-stepped sucrose density gradient (3 mL 60%; 3 mL 35%; 3 mL 20% sucrose solutions) and 10 mL instead of 14 mL of total volume; with the reason behind this was '*the shorter the distance –the more small oil bodies could be accumulated on the flotations top*. The method was modified from Ting *et al*. (1996). Approximately an aliquot of 3 mL of the filtered homogenate from step **I.1** was placed at the bottom of the centrifuge tube (V_{total}= 15 mL), and then layered with 7 ml flotation medium [grinding medium containing 0.4 M **instead of** 0.6 M sucrose] on top as this is presented on Figure 3.

Jurnal Kultivasi Vol. 20 (2) Agustus 2021
ISSN: 1412-4718, eISSN: 2581-138x

The tube was then centrifuged (Heraus Sepatech; Varifuge F) at 22,300 *rpm* at 4 °C for 1 hour (*h*) in order to maximize the yield of big- and small-oil bodies to be collected at the top of the flotation medium.

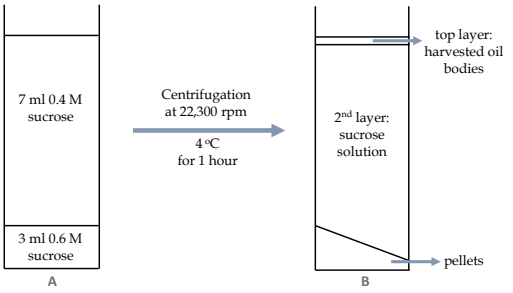

Figure 3. Preparation of two layer sucrose gradient concentration (A); the harvested oil bodies located at the top after centrifugation (modified after Ting *et al.*, 1996).

II) *Analyses of oil bodies diameter via Coulter-Counter*: after centrifugation, the oil body concentrated on top of floating medium was collected with an Eppendorf pipette (V= 10 µL), transferred, and re-suspended in grinding medium. First, we would like to examine the sizes of oil bodies diameter that were able to be collected on the floating top. For this purpose, a manual counting of oil bodies under the light microscope [Zeiss-Axiolab (SIP 41116) with objective lens Plan-Neofluar 44 04 81 integrated with a video camera (RGB TK 1070 from INTAS), and a JVC television produced by INTAS at various magnifications] and dilution was applied when necessary. We only reserved each two genotypes from China, and Germany, that were attributed with the highest and lowest oil content (results not shown here).

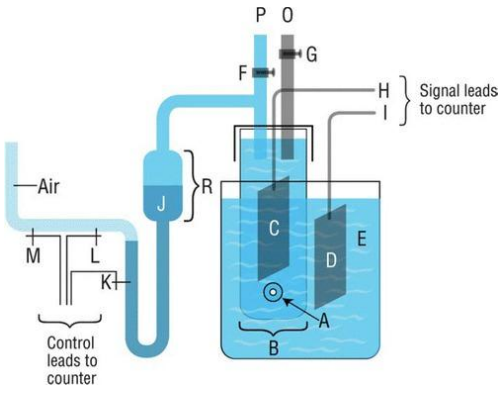

Figure 4. The Multisizer 3 using the principle of Coulter Counter (Graham, 2003)

About 3-ml of aliquot would be further analyzed with the Coulter Counter (Beckman, Germany), for determining the size of oil bodies. The measurement based on the Coulter Counter itself is based on measurable changes in electrical resistance produced by non-conductive particles suspended in electrolyte. The suspended tiny particles were made to flow through a small cylindrical opening or aperture that can be used to measure particles with an overall particle size-range of 0.4 - 1,600 µm. The aperture, itself, separates two electrodes between an electric current flow (Figure 4).

Each particle, which is passed through the aperture *or* in other words, the sensing zone displaced its own volume of conducting liquid, parallelly increasing the impedance of the aperture, and causing a short-term change in the resistance across the aperture. This resistance change could be detected and measured either as a voltage or current pulse. By quantifying the number of pulses and their amplitudes, the information regarding the number of particles and the volume of each individual particle can be obtained. In adjunction to that, the number of pulses detected during measurement is the number of particles measured, and the amplitude of the pulse is proportional to the particle's volume (Khan and Croser, 2004).

The statistical analysis (average, minimum- and maximum values, *Goodness-of-fit* tests for the normal distribution, t-test, F-test, the correlation analyses), and the developed graphics, here, were measured based on the calculation produced by the SAS-program (American Inc.).

Results and Discussion

This study reported the variation of sizes in the oil bodies belong to the Double Haploid genotypes of rapeseed (*Brassica napus* L.) grown on two locations: China and Germany. The isolated oil bodies apparently have a spherical shape containing triacylglycerols (TAG) with diverse diameters ranging from 0.5 – 2.5 µm. Oil bodies in general contain a few major and related unique proteins: oleosins and caleosins, which are unique to the organelles with a 'distinct' three structural domain. Oil bodies are remarkably stable either inside the cell or in isolated preparations and when they are pressed against one another *in-vivo* due to seed desiccation or *in-vitro* after flotation

centrifugation (Tzen and Huang, 1992). Future interest of oil body application is intended as an 'appropriate' vehicle for the production of 'targeted' recombinant proteins or as bio-encapsulation material due to its unique membrane conformation (Peng *et al.,* 2003). Therefore, this study is intended to identify such novel opportunity as a new platform of genetic engineering in oil plant.

Results are divided into three parts.

Established procedure of oil bodies isolation in rapeseed (*B. napus*). Two layer sucrose gradient was successfully to be employed in order to isolate the oil bodies from seeds of rape *(Brassica napus* L.*)*, while, the other one was more suitable for oil bodies isolation from embryos of maturing kernels in maize (*Zea mays*). Prior to the main experiment, we did some modifications from Ting *et al*. (1996), in which the centrifugation time was trialed and varied from 30 up to 270 minutes (*min*.). Then, the collected oil bodies were *pre*-examined under the light microscope, and we revealed that the floating oil bodies in the two layer sucrose gradient medium were visually much better to be analyzed under light microscope. It seemed that, centrifugation period lasted up to an hour was necessary in order to accumulate the oil bodies on the flotation top; particularly those having average size of 1,84 µm in diameter.

Contrastingly, smaller oil bodies with an average size less than 0,79 µm in diameter were still remained at the 2nd layer and hindered to be collected at the top. Interestingly, we revealed that 'oleosins' that play a great role in the stability function of oil bodies (Frandsen *et al*., 2001) and it has been suggested that the entire surface of oil bodies is seemly covered by oleosins. Thus, the profound and compressed oil bodies in the cells would not be coalesce or aggregate (Chuang *et al*., 1996). Nevertheless, our result here suggested that oleosins might have some limitations, in which that they seemly could not endure an extremely long centrifugation and extreme speed as the oil bodies were already deteriorated, afterwards (picture produced by the light microscope not shown).

Analyzes of oil bodies diameter in rapeseed with different oil content via Coulter Counter. The size of oil bodies in rapeseed was varied even within one species in the Double Haploid (DH) population; depending on their oil content. The DH population was derived from the F_1 resulted from a cross between the German cultivar '*Sollux*' and the Chinese one '*Gaoyou*'; both were previously selected as parental lines for high oil content; with the oil content within the DH population varied from 44% to 65% (Zhao *et al*., 2002).

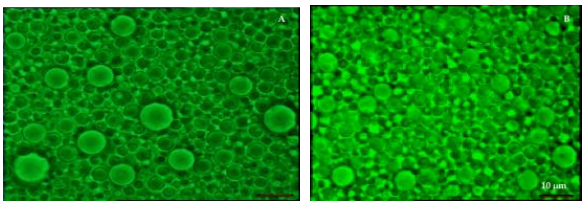

Figure 5. Light microscopy of *B. napus'* oil bodies from genotype with high oil content (A; left**) and low oil content (B) in a DH population cultivated on German environment, note: the other two pictures from the Chinese environment were not able to be presented, here.**

Our result based on the light microscopy showed that oil bodies originated from genotype with high oil content (Figure 5A) had significantly a larger diameter compared to the other one with low oil content (Figure 5B).

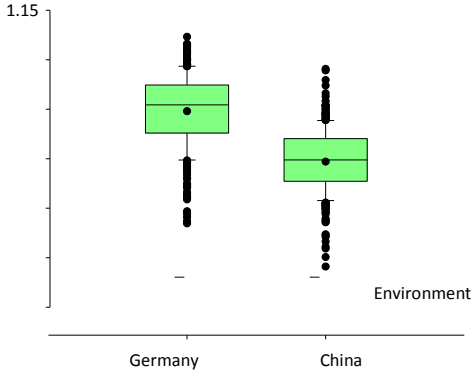

Figure 6. Size distribution of oil bodies diameter from genotypes planted in Germany and China.

Furthermore, the size of oil bodies also varied between the genotypes grown in two environments following the normal size distribution. Those genotypes cultivated on the German environment (N= 284 samples) had an average oil content of 56.94% with a variation of oil bodies size between 0.78 to 1.15 µm (average= 1.05 µm). Meanwhile, those grown on the Chinese environment had a slightly lower average of oil content, namely 49.18% with a variation of oil bodies size 0.75-1.09 µm (average= 1.00 µm) (N= 281 samples) (Figure 6).

Jurnal Kultivasi Vol. 20 (2) Agustus 2021
ISSN: 1412-4718, eISSN: 2581-138x

Result of t-test based on *Kolmogorov-Smirnov* showed that the averages between the two environments was significantly different ($p<0.05$). Furthermore, the Analyses of Variance (ANOVA) also showed that genotypes player a significant role in the variation of oil bodies (F value= 1.31; $p< 0.05$) (Table 1).

Table 1. Analyses of Variance (ANOVA) table revealing the factors affected the average oil bodies size between the two locations in DH population.

Source	F	SS¶	MS§	F-Value	Pr>F
Genotypes	283	0.491	0.002	**1.31**	0.0080
Locations	1	0.355	0.355	**268.26**	*<0.0001*
Error	280	0.371	0.001		
Corrected Total	564	1.226			

SS¶= Sum of Squares; MS§= Mean of Squares

Correlation analyses between oil content and oil body diameter in rapeseed. The variation of oil content within the DH population cultivated in China was in the range of 44% to 54% (average= 49. 18%), meanwhile in Germany was in the range between 45% to 63% (average= 56. 94%). Here, we can conclude that the distinct environment, where the two gentoypes were grown, had played a significant role in influencing the size of oil bodies in DH population (Spasibionek *et al*., 2020), as this was indicated with an F value of 268, 26.

A very highly significant positive correlation between the size of oil bodies in rapeseed and the oil content from those population each cultivated in China and Germany was presented on Figure 7.

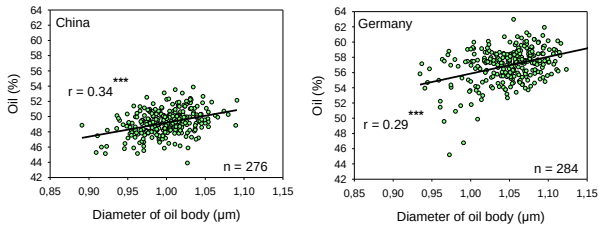

Figure 7. Correlation between oil content vs. oil bodies diameter of DH population cultivated in Germany and China.

The correlation ratios were (r) = 0.34*** and (r) = 0.29*** in China and Germany, respectively. This finding supports the previous report regarding the correlation observed in maize regarded as *Illinois High Oil* (IHO) and *Illinois Low Oil* (ILO) (Ting *et al*., 1996).

Frandsen *et al*. (2001) stated that the general size of oil bodies varies between 0.5 – 2, 5 µm in diameter. Such similar result was being confirmed, here. The differences might be affected, due to the genotype variation although it is unclear, in what way they were being influenced. Second was the distinct locations might have played also a role in the variation of sizes and forms of the DH-genotypes. Furthermore, other nutritional status (agricultural inputs) and environmental factors (sun light intensity, precipitation, humidity) might also influence such variation).

Moreover, the averages of oil bodies diameter; grown in two distinct locations- were slightly different , in which those DH accessions grown on German environment seemed to be more suitable to be cultivated in the next growing season, and based on the practical reason, they are deserved to be further researched in terms of their suitability for the oil extraction process *via* centrifugation. Next questions might be interesting to know whether the phospholipids content might play also a role in affecting the size of oil bodies.

Conclusion

Two layer sucrose gradient was successfully to be employed in order to isolate the oil bodies from seeds of rape *(Brassica napus* L.*)*. The mean diameter of oil bodies size in rapeseed was in the range of 1 µm; with the lowest – and largest ones were 0,12 – 10,30 µm and 0.65- 9,51 with manual counting and Coulter Counter, respectively. There was a highly significant correlation between oil bodies size and oil content, while the big size of oil bodies would be more advantageous in the oil extraction process via centrifugation method. A manipulation of oil bodies in rapeseed via genetic engineering would be advantageous both for: (a) breeding purpose: increasing the oil content, and (b) oil processing: as a more atrractive alternative esp. by the industrial oil processing in rapeseed, which is more environmental friendly and safer for human's consumption.

Acknowledgement

This work received an indirect fund from the German Academic Exchange Service (DAAD) through one of the co-author during his/her

Post Graduate Study at the Georg August Universität in Göttingen, Germany.

Reference

Chuang, R. L. C., J. C. F. Chen, J. Chu, and J. T. C. Tzen, J.T.C. 1996. Characterization of seed oil bodies and their surface oleosin isoforms from rice embyros. J. Biochem. (120): 74-81.

Cunxu, W., F. Qin, Chen, A. Li, Y. Zhang, W. Zhou, and Y. Wang. 2009. Oil body observation in seeds of *Brassica napus* L.. Chinese Journal of Oil Crop Sciences. 31 (4): 445-448.

Frandsen, G. I., J. Mundy, and J. T. C. Tzen. 2001. Oil bodies and their associated proteins, oleosin and caleosin. Physiologia Plantarium (112): 301-307.

Graham, M. D. 2003. The Coulter Principle: Foundation of an Industry. JALA: Journal of the Association for Laboratory Automation, 8(6):72-81

Huang, A. H. C. 1996. Oleosins and oil bodies in seeds and other organs. Plant Physiol. (110): 1055-1061.

Jiang, P. L. and J. Tzen. 2010. Caleosin serves as the major structural protein as efficient as oleosin on the surface seed oil bodies. Plant Signaling & Behaviour. 5 (4): 447-449.

Khan T. N. and J. S. Croser. 2004. PEA Overview. Encyclopedia of Grain Science. Pages 418-427

Kumar, S. and W. F. Thompson. 2010. Plant Genome Engineering Using Zinc Nucleases in Molecular Techniques in Crop Improvement, Jain, S.M. and Brar, D.S. (*eds.*) ,doi: 10.1007/978-90-481-2967-6_24, Springer Science and Business Media B.V. , pp. 579-590

Millichip, M., A. S. Tatham, F. Jackson, G. Griffiths, P. R. Shewry, and A. K. Stobart. 1996. Purification and characterization of oil-bodies (oleosomes) and oil-body boundary proteins (oleosins) from the developing cotyledonds of sunflower (*Helianthus annuus* L.). Biochem. J. (314): 333-337.

Peng, C. C., I. Lin, C. Lin, and J. T. C. Tzen. 2003. Size and stability of reconstituted sesame oil bodies. Biotechnol. Prog. (19): 16233-1626.

Ren, J., P. Wu, B. Trampe, X. Tian, T. Lubberstedt, and S. Chen. 2017. Novel technologies in dhoubled haploid line development. Plant Biotechnology Journal 15, 1361-1370. https://onlinelibrary.wiley.com/doi/epdf/10.1111/pbi.12805

Spasibionek, S., K. Mikolajczyk, H. Ćwiek-Kupczyńska, T. Piętka, K. Krótka, *et al*. 2020. Marker assisted selection of new high oleic and low linolenic winter oilseed rape (*Brassica napus* L.) inbred lines revealing good agricultural value. PLOS One 15 (6):e0233959. https://doi.org/10.1371/journal.pone.0233959

Strohm, K. 2010. Description of the typical farm DE360OW, Germany. http://www.agribenchmark.org/cash-crop/sector-country-farm-information/country-profiles/germany.html; accessed 27 Februari 2021

Ting, J. T. L., K. Lee, C. Ratnayake, K. A. Platt, R. A. Balsamo, and A. H. C. Huang. 1996. Oleosin genes in maize kernels having diverse oil contents are constitutively expressed independent of oil contents. Planta (199): 158-165.

Tzen, J. T. C. and A. H. C. Huang. 1992. Surface structure and properties of plant seed oil bodies. The Journal of Cell Biology (117:2): 327-335.

Wäsche, A. 2001. Simultäne Öl-und Proteingewinnung bei Raps. Genehmigte Dissertation von der Fakultät III-Prozesswissenschaften der Technischen Universität Berlin. Berlin, Germany.

Zhao, J. 2002. QTLs for oil content and their relationships to other agronomic traits in an European x Chinese oilseed rape population. Online dissertation at the University of Göttingen: pp. 28.