Journal of Cereal Science 101 (2021) 103182

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

A first step towards *in vitro* cultured cereals

Odd-Arne Olsen

Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, 1430, Norway

Cellular agriculture is emerging as a branch of biotechnology addressing issues of environmental impact and expanding the variety of food sources in order to create novel foods.

Animal meat is cultured *in vitro* using appropriate cell lines and cell culture practices. Although much development work is needed before cultured meat can provide significant contributions to the food supply chain, promising progress is under way (for a recent review, see Post et al., 2020).

The purpose of this text is to raise discussion on whether similar *in vitro* culture of cereal endosperm is technically achievable, and if so, for what end use. Already, small scale maize endosperm organ cultures are available for basic science studies and plant breeding research.

Worldwide harvest of cereal grains from maize, wheat, rice, sorghum, barley and oats amounts to 2.8 million metric tons, providing substantial input of food and calories for humans as well as raw material for food industries. Annual improvement of yield and quality of cereals is delivered by traditional breeding as well as transgenic approaches. Still, yield improvement is struggling to keep pace with the demand from a growing world population. In addition, the use of cereal grains as food raw material is limited by their protein composition, which is difficult to alter without reducing grain yield. A well-known example of this challenge is the failed plant breeding effort to enhance grain content of the essential amino acid lysine for enhancing the diet of monogastric animals, including humans (Fereirra et al., 2005). Transgenic approaches to alter other grain nutrient properties, on the other hand, have been more successful, including the development of golden rice with enhanced content of Vitamin A to fight blindness in children (Beyer et al., 2002).

1. Structure of cereal endosperm

Cereal grains consist of four tissues; starchy endosperm forming the central body of the grain, an aleurone layer surrounding the starchy endosperm, the basal transfer cell layer and the embryo surrounding cell layer (Fig. 1A). For a recent review see (Olsen, 2020). The latter two cell types contribute little to no nutritional value to cereal grains.

The starchy endosperm accounts for approximately 80% of the grain dry weight and consists mainly of starch granules and storage proteins, including gluten, a main determinant of baking quality in wheat. Other components, such as lipids and dietary fibers, affect health and food-processing characteristics and are present in smaller amounts (Tosi et al., 2018). The starchy endosperm has a high capacity for protein synthesis, expressing a limited number of genes in high copy numbers (Pfeifer et al., 2014).

Aleurone cells have thick cell walls that, similar to those of the starchy endosperm, contain mostly beta glucans and arabinoxylan (see for example (Brouns et al., 2012) and references therein); sugars represent approximately 40% of the aleurone dry weight. In wheat, the aleurone layer contains 15% of the total grain proteins and as much as 30% of total grain lysine. Aleurone cells are also a rich source of nutrients, such as minerals, phytates, vitamins B including niacin, folate and plant sterols. For example, 80% of total wheat grain niacin is in aleurone cells, together with considerable amount of other vitamins B and approximately 50% of the total wheat grain mineral content. Milling and dry-fractionation techniques have allowed for full-scale separation of aleurone cells from the wheat bran, yielding a concentrate rich in bioactive compounds (Brouns et al., 2012) as well as the elaboration of a novel flour that has a high contents of bioavailable folate (Fenech et al., 1999).

1.1. *In vitro culture of endosperm*

The first successful protocol for organ culture of young dissected maize kernels was developed in 2006 based on a medium containing high (15%) sucrose (Gruis et al., 2006), cells differentiating into aleurone and starchy endosperm cells. This was an important achievement since earlier attempts to culture maize endosperm *in vitro* had resulted in undifferentiated cells (Shannon and Battey, 1973). Interestingly, when cultured on high concentration of sucrose, developing endosperms form cell-congregates with multiple spherical bodies or “mini-endosperms”, consisting of a core of starchy endosperm cells surrounded by a single layer of aleurone cells (Fig. 1 B, C). The maize endosperm, and likely all cereals, is programmed to differentiate aleurone cells in the outermost cell layer, irrespective of the shape or size of the body. Gene expression

E-mail address: odd-arne.olsen@nmbu.no.

https://doi.org/10.1016/j.jcs.2021.103182
Received 14 October 2020; Received in revised form 2 February 2021; Accepted 4 February 2021
Available online 9 February 2021

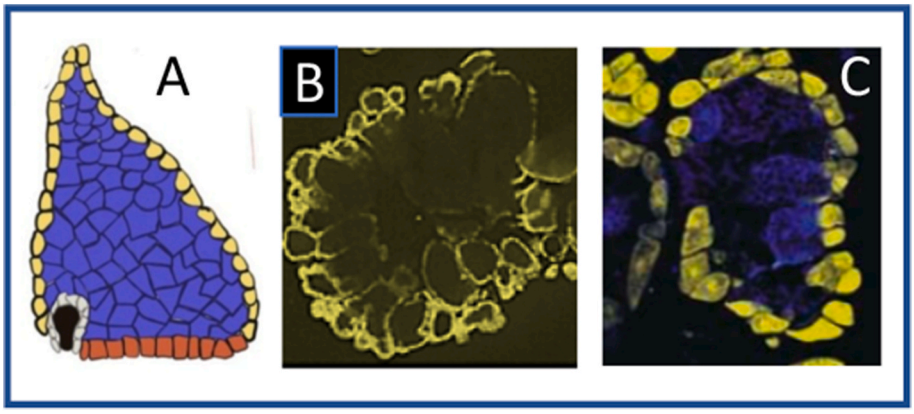

Fig. 1. The structure of plant grown and *in vitro* grown endosperm. A-The starchy endosperm consists of large cells forming the core body of the grain (blue) surrounded by a layer of aleurone cells (yellow). Transfer cells form a basal layer of the endosperm (red) and the cells of the embryo surrounding region encases the embryo at an early developmental stage of the grain. B- Cultured endosperms consist of numerous spherical bodies referred to as mini-endosperm with a surface layer of aleurone cells (yellow; starchy endosperm cells are not colored in this picture). C- Mini-endosperm with aleurone cells (yellow) and interior starchy endosperm cells (cyan). The colors are from expression of yellow and cyan fluorescent proteins driven by aleurone- and starchy endosperm-specific promoters, respectively. For details, please see (Gruis et al., 2006). Figures are redrawn after (Olsen, 2020). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

as revealed by transcript profiling in cultured endosperms follows closely that of in planta-grown endosperms, demonstrating that cultured endosperm development is regulated in space and time similarly to plant grown-endosperms, even in the absence of signal inputs from the mother plant. In fact, developmental endosperm defects seen in some mutants can be recapitulated in *in vitro* cultured endosperms. For example, mutations in the *Dek1* gene, which is necessary for aleurone cell fate specification, eliminate aleurone cells from cultured endosperm as well, resulting in cultures with only starchy endosperm cells (Gruis et al., 2006). In addition, it is also possible to genetically transform *in vitro* cultured endosperm either by biolistic bombardment or Agrobacterium-mediated transformation, offering the possibility to tailor the composition of cultured endosperm to novel uses (Reyes et al., 2010; Dinga et al, 2021).Transfer cells, and most likely embryo surrounding cells, are absent from cultured endosperm (Olsen, 2020).

1.2. Candidate gene assays using endosperm organ cultures

The unique property of endosperm organ *in vitro* cultures is their ability to maintain the organ-level differentiation, and hence transcriptome profile of in planta endosperm. As such, the system offers a fast track to study expression of genes of interest in maize endosperm that would otherwise have to be studied in transgenic plants. Although maize transformation is feasible at high frequencies in specialized labs, the process may take up to a year. The immediate utility is therefore to enable plant breeders and plant biologists to determine the effect of candidate genes in endosperms without the need to create transgenic plants (Reyes et al., 2010). An additional benefit is that gene characterization can be carried out under contained conditions without release of transgenic plants to the environment. The opportunity to express transgenes in differentiated endosperm cells *in vitro* also offers a unique ability to study mechanisms of endosperm cell fate specification and differentiation without intact plants.

Developing similar endosperm organ cultures for the other cereals would offer similar advantages. For example, wheat endosperm cultures could facilitate initial testing of the effect on baking quality of gluten proteins, bypassing the need for transgenic plants. As such, the system would enable high throughput testing of the large number of gluten proteins known to carry epitopes provoking coeliac response in susceptible patients (Juhàsz et al., 2018). To this author's knowledge, no systematic effort has been reported to establish endosperm organ cultures for other cereal species than maize.

1.3. Large scale endosperm in vitro organ cultures?

Large scale cultures of endosperm has the potential to facilitate production of specialized cereal products normally produced in fields, for example gluten proteins without coeliac-inducing epitopes as mentioned above. Whether or not this is feasible in the foreseeable future is unknow. Efforts to grow maize endosperm organ cultures had lasted for almost fifty years when the discovery was made that high sucrose induce endosperm differentiation *in vitro* (Gruis et al., 2006). A systematic approach to large scale cultures could follow two approaches, first, as is already under way, by elucidating the complete developmental program of endosperm (Pfeifer et al., 2014, Olsen, 20120), potentially identifying ways to lock development into a state with high mitotic activity and optimal expression of targeted products. Alternatively, a "blind" approach could expose the current maize cultures to various treatments hoping to trigger such cultures.

2. Conclusion

In this author's opinion, *in vitro* organ endosperm cultures do not have the potential to replace field grown cereals. In its current form, high throughput testing of maize candidate genes for endosperm engineering can be carried out without release of transgenes to the environment. Expansion to other cereals would permit similar assays for e.g. wheat, barley and rice. Future development of high volume endosperm organ cultures could produce existing and novel cereal endosperm products. Whether or not such cultures could be more productive than bacterial, yeast or algal culture systems remains to be seen.

Declaration of competing interest

There is no conflict of interest involved in the work presented in the submitted manuscript.

Acknowledgement

Marisa Otegui is thankfully acknowledged for valuable discussion and comments.

References

Beyer, P., Al-Babili, S., Ye, X., Lucca, P., Schaub, P., Welsch, R., Potrykus, I., 2002. Golden rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J. Nutr. 132, 506S–510S.

Brouns, F., Hemery, Y., Price, R., Anson, N.M., 2012. Wheat aleurone: separation, composition, health aspects, and potential food use. Crit. Rev. Food Sci. Nutr. 52, 553–568.

Dinga, Xinxin, Zhang, Xiaoguo, Olsen, Odd-Arne, Oteguia, Marisa S., 2021. Transient expression of fluorescently tagged proteins in developing maize aleurone cells. MethodsX 8, 101446. https://doi.org/10.1016/j.mex.2021.101446.

Fenech, M., Noakes, M., Clifton, P., Topping, D., 1999. Aleurone flour is a rich source of bioavailable folate in humans. Human Nutr. Metabol. 129, 1114–1119.
Ferreira, R.R., Varis, V.A., Meinhardt, L.W., Lea, P.J., Azevedo, R.A., 2005. Are high lysine cereal crops still a challange? Braz. J. Med. Biol. 38, 985–994.
Gruis, D.F., Guo, H., Selinger, D., Tian, Q., Olsen, O.-A., 2006. Surface position, not signaling from surrounding maternal tissues, specifies aleurone epidermal cell fate in maize. Plant Physiol. 141, 898–909.
Juhász, A., Belova, T., Florides, C.G., Maulis, C., Fischer, I., Gell, G., Birinyi, Z., Ong, J., Keeble-Gagnère, G., Maharajan, A., Ma, W., Gibson, P., Jia, J., Lang, D., Mayer, K.F. X., Spannagl, M., IWGSC Tye-Din, J.A., Appels, R., Olsen, O.-A., 2018. Genome mapping of seed-borne allergens and immunoresponsive proteins in wheat. Sci. Adv. 4 https://doi.org/10.1126/sciadv.aar8602 eaar8602–24.
Olsen, O.-A., 2020. The modular control of cereal endosperm development. Trends Plant Sci. 35 (3), 279–290. https://doi.org/10.1016/j.tplants.2019.12.003, 1–12.
Pfeifer, M., Kugler, K.G., Sandve, S.R., Zhan, B., Rudi, H., Hvidsten, T.R., International Wheat Genome Sequencing Consortium, Mayer, K.F.X., Olsen, O.-A., 2014. Genome interplay in the grain transcriptome of hexaploid bread wheat. Science 345, 1250091–1250091.
Post, M.J., Levenberg, S., Kaplan, D.L., Genovese, N., Fu, J., Bryant, C.J., Negowetti, N., Verzijden, K., Moutsatsou, p., 2020. Scientific, sustainability and regulatory challenges of cultured meat. Nat. Food 1, 403–415. https://doi.org/10.1038/s43016-020-0112-z, 2020.
Reyes, F.C., Sun, B., Guo, H., Gruis, D.F., Otegui, M.S., 2010. Agrobacterium tumefaciens-mediated transformation of maize endosperm as a tool to study endosperm cell biology. Plant Physiol. 153, 624–631.
Shannon, J.C., Batey, J.W., 1973. Inbred and hybrid effects on establishment of in vitro cultures of Zea mays L. endosperms. Crop Sci. 15, 491–493.
Tosi, P., He, J., Lovegrove, A., Gonzáles-Thuillier, I., Penson, S., Shewry, P.R., 2018. Gradients in compositions in the starchy endosperm of wheat have implications for milling and processing. Trends Food Sci. Technol. 82, 1–7.