AARHUS
UNIVERSITET
DCA - NATIONALT CENTER FOR FØDEVARER OG JORDBRUG

Til Landbrugsstyrelsen

Følgebrev

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Vedr. "Historiske erfaringer med anvendelse af tilfældig in vitro mutagenese i dansk planteforædling".

Landbrugsstyrelsen har i en bestilling, sendt den 10. juni 2020, bedt DCA – Nationalt Center for Fødevarer og Jordbrug – om at lave et kort notat, på engelsk, som redegør for historikken og erfaringerne med anvendelse af "tilfældig in vitro mutagenese" inden for forskning og dansk planteforædling. Notatet skal sammen med bl.a. et bidrag fra de danske planteforædlere indgå i den danske besvarelse af en henvendelse fra EU-Kommissionen.

Besvarelsen i form af vedlagte notat er udarbejdet af Seniorforsker Per Gregersen og Professor Henrik Brinch-Pedersen, begge fra Institut for Agroøkologi ved Aarhus Universitet samt Professor Torben Asp fra Center for Kvantitativ Genetik og Genomforskning ved Aarhus Universitet.
Seniorforsker Inger Bæksted Holme fra Institut for Agroøkologi ved Aarhus Universitet, har været fagfællebedømmer.

Besvarelsen er udarbejdet som led i "Rammeaftale om forskningsbaseret myndighedsbetjening mellem Planteproduktion 2020- 2023".
Miljø- og Fødevareministeriet og Aarhus Universitet" under ID 1.21 i "Ydelsesaftale

Venlig hilsen

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A scientific opinion on in vitro random mutagenesis techniques

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Background

The European Commission has requested memberstates for information on the development and application of in vitro mutagenesis techniques on certain varieties of agricultural species. As a consequence, the Ministry of Environment and Food of Denmark has on the 10.06.2020 requested DCA - Danish Centre For Food And Agriculture, Aarhus University, to provide a brief presentation, in English, of the history and experience of using "random in vitro mutagenesis" in research and Danish plant breeding.
The note has been produced under the contract re-science based policy advice between the Ministry of Environment and Food of Denmark and Aarhus University and it is understood that this note or parts hereof may be forwarded to the European Commission as part of the Danish reply.

Reply

1.1 *In vivo* versus *in vitro* mutagenesis

The definition of *in vitro* mutagenesis made in the French Conseil d'Etat decision ("subjecting plant cells cultivated *in vitro* to chemical or physical mutagenic agents") leads to ambiguous interpretations of the difference between *in vivo* and *in vitro* mutagenesis, mainly due to the lack of precision in what is covered by "plant cells cultivated *in vitro*". The *in vivo* mutagenesis is defined as mutagenesis made on whole plants or parts of plants. However, with respect to "parts of plants" (which could take many forms, e.g. seeds, stem fragments, dissected embryos, meristematic tissues, pollen etc.), these would often be handled through *in vitro* techniques (commonly designated by the wider term "tissue cultures") (Suprasannaa *et al.*, 2011). These techniques are well-established techniques used at a broad scale in plant research and breeding for plant propagation and they have track records going back many decades. However, *in vitro* techniques are not always necessary to obtain mutants, e.g. with stem fragments, and, hence, the borderline between *in vivo* and *in vitro* mutagenesis, as defined by the French Conseil d'Etat, is blurry. Strictly speaking, the definition of *in vitro* mutagenesis would only cover approaches where mutagenesis is made on isolated protoplasts or suspensions of microspore cells. Mutant genotypes obtained with these techniques, e.g. for creating herbicide resistance, have as well been obtained with mutagenesis on intact plant parts (Tan and Bowe, 2011). In the end, any regenerated mutant plant would rely on mutagenesis of single cells that go into the germ line of the plant, disregarding whether the mutagenesis is

made on a plant part or on cell cultures. This also makes the distinction between *in vivo* and *in vitro* mutagenesis appear arbitrary, if not futile.

1.2 History of *in vitro* mutagenesis

Keeping it to the strict definition of *in vitro* mutagenesis of cell cultures, evidence from the literature on these techniques can be traced back to at least the 1980's. As examples, Negrutiu (1981) described mutagenesis of tobacco protoplasts by UV or chemical treatments followed by regeneration of mutant plants, and Swanson *et al.* (1988) described the mutagenesis of microspores by chemical treatment and regeneration of mutated herbicide-resistant plants of *Brassica napus*. From the 1990's and 2000's there are several reports on mutagenesis *in vitro* of microspores and haploid cells in general (reviewed by Szarejko, 2011). With a broader definition of *in vitro* mutagenesis including procedures where any sort of *in vitro* growth of plant tissues in combination with mutagenesis has been applied, the techniques can also be traced back to at least the 1980's (e.g. Roest *et al.*, 1981). In summary, *in vitro* mutagenesis in plants is definitely developed before March 12th, 2001.

1.3 Danish plant breeding

There are no published information on the use of *in vitro* mutagenesis in Danish plant breeding, and according to our information Danish plant breeders of cereals, potatoes, and grasses have not bred or marketed cultivars with traits based on this approach during the last two decades. No information is available on whether cultivars bred abroad, but marketed and grown in Denmark are based on *in vitro* mutagenesis techniques. Tissue culture techniques are commonly used in plant breeding (double haploid generation, meristem propagation, callus cultures etc.), but the intended use of *in vitro* mutagenesis in this context is nevertheless not currently used in Danish plant breeding. However, the interest in using mutagenesis as a tool in plant breeding, at least for agricultural crops, is increasing again after experiencing a decrease since the 1980's (Brinch-Pedersen *et al.*, 2018).

A review of officially released mutant varieties reports that from a total of 1,847 accessions of the FAO/IAEA Mutant Varieties Database (http:// www-mvd.iaea.org), crop species are represented by 1,357 officially released mutant cultivars and ornamental and decorative plant species by 490 mutant varieties (Roychowdhury and Jagatpati, 2013). Among the cereals (869 mutant varieties), rice (333) ranks first, followed by barley (261), bread wheat (147), maize (49), durum wheat (25), and others (54). Accordingly, mutants must be regarded as an integrated part of breeding materials and commodities.

1.4 With respect to implementation of Directive 2001/18 on March 12th 2001

The basics of the *in vitro* mutagenesis (using plant cells) was developed already in the 1980's. It has particularly been used for the development of herbicide resistance in rapeseed (reviewed by Tan and Bowe, 2011), probably because microspore cultures are easy to establish in this crop, and the selection of mutated plants is easy to perform following treatment of the microspore cultures with herbicide before full regeneration of plants. On a broader scale, *in vitro* techniques in combination with mutagenesis (also including plant parts propagated *in vitro*) also trace back to at least the

1980's (e.g. Roest *et al.*, 1981). As examples of *in vitro* mutagenesis before 2001 in species of relevance for Danish conditions, the following can be mentioned:

Rapeseed

- Imidazolinone-tolerant rapeseed microspores of the oilseed rape variety Topas were mutagenized using ethyl nitrosourea (Swanson et al., 1988).
- In 1995, the mutant rapeseed cell suspension culture line C20 was created with a Trp-574-Leu single amino acid change leading to sulfonylurea resistance (Hattori et al. 1995).

Maize

- Imidazolinone-tolerant maize was generated in 1982 where tissue culture selection of cell callus of the maize hybrid A188 × B73 with imazaquin resulted in several imidazolinone-tolerant lines (Shaner et al., 1996).

1.5 Conclusions

The *in vivo*/-*vitro* definition as stated by the French Conseil d'Etat is ambiguous with respect to the material used. In a broader sense, where the definition includes the use of any *in vitro* technique, the approach has been widely used internationally, but is currently not used by Danish plant breeders to breed agricultural crops. The *in vitro* techniques have a long track record, tracing back to at least the 1980's, thus they were clearly developed before March 12th 2001. They have mainly been used (with cell cultures) in rapeseed and maize, two species that are not bred in Denmark, but widely grown here using cultivars bred abroad.

1.6 References

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