Scientia Horticulturae 130 (2011) 1–8

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Review

In vitro culture of endosperm and its application in plant breeding: Approaches to polyploidy breeding

Yoichiro Hoshino[a,b,*], Tomomi Miyashita[b], Thuruthiyil Dennis Thomas[a,c]

[a] Field Science Center for Northern Biosphere, Hokkaido University, Kita 11, Nishi 10, Kita-Ku, Sapporo 060-0811, Japan

[b] Course in Agro-Ecosystem Science, Division of Biosphere Science, Graduate School of Environmental Science, Hokkaido University, Kita 11, Nishi 10, Kita-Ku, Sapporo 060-0811, Japan

[c] Department of Botany, St. Thomas College, Pala Arunapuram (P.O.) 686574, Kottayam (D.T.), Kerala, India

ARTICLE INFO

Article history:
Received 29 April 2011
Received in revised form 19 June 2011
Accepted 26 June 2011

Keywords:
Endosperm culture
Plant regeneration
Polyploidy breeding
Triploid

ABSTRACT

This review article provides an overview of plant regeneration from endosperm to produce polyploid plants. We discuss the endosperm-ploidy levels and its applications in plant breeding. Representative cases of successful endosperm culture and prospects of endosperm culture are described. In the first part of the review, i.e., the background of endosperm culture, we discuss the importance of triploid plants, meaning plants derived from the endosperm of diploid plants. A brief history of endosperm culture is also provided, and a description of plant regeneration systems from the endosperm is given. The influence of culture medium on callus induction and regeneration is indicated. Finally, prospects for endosperm culture are proposed, and novel approaches for polyploidy breeding using endosperm culture are described.

Contents

1. Introduction

In diploid plants, the endosperm is a triploid (i.e., having 3 sets of chromosomes) tissue as a result of double fertilization, which is a unique process in higher plants. During the fertilization process, one of the male gametes fuses with the egg to form a zygote, which later forms the embryo; the other male gamete fuses with the central cell, which contains 2 haploid nuclei. This second fusion frequently results in a triploid structure, the endosperm. Hence, the endosperm is formed as a result of double fertilization and triple fusion (i.e., fusion between 3 different haploid nuclei, 1 from

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP (BA), benzylaminopurine; CH, casein hydrolysate; CM, coconut milk; GA_3, gibberellic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog medium; NAA, 1-naphthaleneacetic acid; YE, yeast extract.

* Corresponding author at: Field Science Center for Northern Biosphere, Hokkaido University, Kita 11, Nishi 10, Kita-Ku, Sapporo 060-0811, Japan.
Tel.: +81 11 706 2857; fax: +81 11 706 2857.
E-mail addresses: hoshino@fsc.hokudai.ac.jp, hoshino@exfarm.agr.hokudai.ac.jp (Y. Hoshino).

0304-4238/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.06.041

the paternal and 2 from the maternal side) and is present in all angiosperm families except Orchidaceae, Podostemaceae, and Trapaceae. Earlier studies suggested that the endosperm functions as a nutritive tissue, because growth and development of the embryo depends on the presence of the endosperm (Brink and Cooper, 1947; Raghavan, 1966). Moreover, the endosperm persists in some seeds (like cereals) as a reserve food. The endosperm represents about 60% of the world's food supply (Berger, 2003). When the endosperm fails to develop properly, abortion of the embryo results (Vijayaraghavan and Prabhakar, 1984). Endosperm may be fully utilized by the developing embryo (non-endospermous), or it may persist in mature seeds (endospermous).

In cereal crops, the endosperm shows an accumulation of seed storage proteins that are useful as food (Kawakatsu and Takaiwa, 2010). Protein synthesis and nutrient transport have been analyzed. Coconut water or coconut milk, which are media with a high nutritive value, were used to promote successful tissue culture in *Datura* embryos (Van Overbeek et al., 1941). Thereafter, plant growth regulators such as cytokinins have been found in coconut water (Letham, 1974). Thus far, coconut water has been utilized for tissue cultures in recalcitrant plant species (Thorpe, 2007). Thus, these aspects of endosperm have been studied. In addition, a specific characteristic of endosperm is its ploidy level, which is triploid in diploid plant species.

One of the most important characteristics of triploid plants is seed sterility, and hence, the seed sterility is unfavorable for plants whose seeds are used commercially. However, triploids are of significant importance in trees and shrubs that are important for biomass and soil conservation, because they promote vegetative growth by preserving huge amounts of photosynthetic energy normally channeled to seed and fruit production. Similarly, seedlessness is used to increase the quality of several fruits, like banana, papaya, grapes, apple, etc. According to Morinaga and Fukushima (1935), triploids are more vigorous than diploids. In some plants, like *Miscanthus sinensis*, seed-sterile triploids have been grown to prevent seed dispersal in the environment (Petersen et al., 2002).

Like triploid plants, polyploid plants have been incorporated into breeding programs and practical cultivation. Polyploid plants, for example, tetraploids, can be produced by the chemical treatment (colchicine, oryzalin, etc.) of diploid plants. Moreover, other ploidy levels can be obtained by crossing different ploidy levels. In this study, we propose the usefulness of triploid plants produced by endosperm culture, when further ploidy levels are produced through crossing with other ploidy levels. Furthermore, we discuss the possible endosperm culture from ovules after crossing plants with different ploidy levels.

2. Advantage of producing triploids from the endosperm

2.1. History and applications

Polyploid production has been utilized in breeding several crops. Polyploid plants are generally expected to have enlarged organs. In addition, polyploid plants exhibit disease resistance, delayed flowering, or lower fertility in some cases. These phenotypes are considered to be favorable traits. For chromosome doubling, treatment with chemicals such as colchicine, pronamide, trifluralin, oryzalin, and amiprophos methyl have been investigated (Wan et al., 1991). These chemicals inhibit cell division after chromosome doubling, which results in polyploid cell formation. Chromosome doubling has occurred as a result of these chemical treatments, and higher ploidy plants can be produced.

Triploid plants are traditionally produced by crossing a diploid plant with an induced tetraploid plant. The tetraploid plant is produced from a normal diploid plant by the chemical treatments mentioned previously. However, the cross often results in reduced seed setting compared to diploid × diploid crosses (Sikdar and Jolly, 1995). Moreover, seed germination and seedling survival are very low. Despite these difficulties, some scientists have successfully produced triploid plants through diploid × tetraploid crosses. Some plant varieties developed and released for agricultural purposes are described in the following sections.

3. Conventional triploid utility

3.1. Cassava (Manihot esculenta)

Cassava (*Manihot esculenta* Crantz) is an important root crop that is cultivated in tropical countries and propagated by stem cuttings. It is generally known as a poor man's crop and has become a subsidiary food in many countries. Cassava is also exploited as a raw material for starch-based industries and as cattle feed (Ghosh, 1991). There is great potential for improving the starch yield of this industrially important crop. Cultivated cassava has a diploid number of chromosomes ($2n = 36$) and is highly heterozygous and cross-pollinated. Among artificially produced polyploids, triploids have a higher yield and higher starch potential (Jos et al., 1987; Sreekumari and Jos, 1996).

The first triploid cassava variety 'Sree Harsha' released in 1996 (Sreekumari et al., 1999) was a cross between natural diploid and induced tetraploid plants. The use of a female diploid plant yielded better results than reciprocal crosses. The characteristics include vigorous, non-branching short plants with broad, thick, dark green leaves. Compact roots yield 35–40 t/ha. Even though the crop duration is 10 months, because of its bulky nature the crop can be harvested even after 7 months without any yield loss or starch reduction in the roots.

The triploid cassava has a number of desirable features compared to its diploid counterparts. These include higher yield, a higher harvest index, increased dry matter and starch content in the roots, rapid bulking, early harvestability, shade tolerance, and tolerance to cassava mosaic virus. The triploid cassava combines a high yield with outstanding culinary quality, thereby making it a favorite both industrially and domestically.

3.2. Watermelon

Cushman et al. (2003) evaluated the field performance of various triploid (seedless) watermelon varieties. The 'Vertigo' variety produced the highest yields of marketable melons (41,000 lb/acre and 2270 melons/acre). 'Vertigo' also produced the lowest yields of early melons (8200 lb/acre and 450 melons/acre). Cultivar SWS 4930 produced significantly larger melons (20.6 lb) than all other varieties except 'Seedless Sangria' and SR 8026. The average weight of the 2 oval-shaped "Cooperstown" and "Triple Crown" cultivars was lower than that of the elongated cultivars. Values for soluble solids concentration, hollowheart, and rind necrosis were not significantly different among any of the cultivars tested.

3.3. Little gourd (Coccinia grandis)

A promising triploid variety of *Coccinia grandis* was developed by Suresh Babu and Rajan (2001). The tender fruits of this crop are cooked as a vegetable. The plant has several medicinal properties also. The fruit contains appreciable amounts of iron, vitamin A, and vitamin C. The triploid plants were produced by crossing colchicine-induced tetraploid with a normal diploid parent. Although the fruit set was observed in all such crosses, the seeds per fruit were 2.4%. Morphologically, the triploid plants more or less resembled the diploid, but the significant features of the triploid were its increased fruit size, lower astringency, vigorous growth, and higher yield.