Chapter 12
Albinism in Microspore Culture

A.M. Torp and S.B. Andersen

Abstract Frequent formation of albino plants from *in vitro* cultured microspores is a particular problem for chromosome doubled haploid production in cereals and grasses. The phenomenon was first thought to be associated with maternal inheritance of plastids visualized by large deletions and rearrangements of plastid genomes in albino plants. Subsequently interests have changed to inactivation of plastid ribosomes, which has been shown to create albino phenotypes *in vitro*. A considerable knowledge on genetic regulation of the trait is used in this chapter to forward a hypothesis that the stressful *in vitro* conditions in these cultures make the plants fight their own plastids with antibiotic like compounds.

Keywords Albinism, plastid deficiency, haploids

Introduction

The discovery of the ability of plant microspores to develop directly into haploid plants *in vitro* (Guha and Maheshwari 1964; Nitsch and Nitsch 1969) spurred a great amount of optimism and experimental enthusiasm. The general application of the technique, however, met obstacles due to low *in vitro* response in most species and for some of the most important cereals, in addition, many completely white albino plants were regenerated (De Buyser and Henry 1979; Wang et al. 1974).

High frequency of albino plant formation in anther and microspore cultures is a general phenomenon in most cereals like wheat (Andersen et al. 1987), barley (Knudsen et al. 1989), rice (Guiderdoni et al. 1992), rye (Immonen 1999), oat

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(Kiviharju and Pehu 1998) and in several grasses like ryegrass (Olesen et al. 1988) and timothe (Abdullah et al. 1994). These white plants contain plastids arrested in development towards functional chloroplasts (Caredda et al. 1999, 2000; Sun et al. 1974) and therefore cannot grow without the sugar containing media of *in vitro* cultures (Sun et al. 1974).

An early hypothesis connected such albino plant formation with the maternal inheritance of plastids observed for most angiosperm plant species (Vaughn et al. 1980). Such ideas had strong support when it was demonstrated that albino plants from microspores of several different cereal species had deletions and reorganizations in their plastid (ptDNA) genomes (Day and Ellis 1984; 1985; Harada et al. 1991; Hofinger et al. 2001). Targeted inactivation of selected plastid genes of tobacco i.e. *rpo* genes encoding the plastid RNA polymerase, has been shown to generate albino phenotypes in tobacco (Allison et al. 1996; De Santis-Maciossek et al. 1999; Serino and Maliga 1998). Thus it is now well documented that such deletions of parts of the plastid genome can generate the albino phenotype *in vitro* at least in some plant species though the relationship with maternal inheritance has not been documented.

However, not all androgenic albino plants carry deletions in their ptDNA genome. Albino plants have been found which show restriction patterns indistinguishable from those of intact ptDNA genomes in wheat (Day and Ellis 1984; Hofinger et al. 2001) barley (Dunford and Walden 1991) and rice (Harada et al. 1991). Furthermore, Hofinger et al. (2001) observed transcription from all plastid DNA regions in these albino plants. Thus doubt has been raised as to what extend plastid genome deletions and rearrangments can indeed explain the high frequency of albino plant regenerants in many cereal microspore cultures (Hofinger et al. 2001).

Similar alterations in transcript pattern and translation levels have been found in plastids of albino plants with and without ptDNA deletions. Transcript levels of plastid encoded genes for photosynthetic proteins and ribosomal RNA were generally heavily reduced in albino plants relative to green plants (Ankele et al. 2005; Dunford and Walden 1991; Hofinger et al. 2001). In contrast, transcript levels of plastid encoded house-keeping genes were largely unaffected (Hofinger et al. 2001) or significantly elevated in such albino plants (Ankele et al. 2005). In addition, Sun et al. (1979) and Hofinger et al. (2001) found one of more plastid encoded photosynthetic or ribosomal proteins to be absent or strongly reduced in rice and wheat microspore derived albino plants. These results have led to the suggestion that the androgenic albino plants lack functional plastid ribosomes as this would explain both the translation deficiencies and the modified transcript pattern (Hofinger et al. 2001).

Deficiency of plastid ribosomes in cereals are non-lethal under *in vitro* conditions and can be caused by different factors. Recessive mutations in nuclear encoded genes leading to plastid ribosome deficiency in the affected tissue is known from both the barley mutant *albostrians* (Knoth and Hagemann 1977) and the maize mutants *iojap* (Shumway and Weier 1967; Thompson et al. 1983) and *ppr2* (Williams and Barkan 2003). *Ppr2* is thought to be involved in synthesis or assembly of one or more components of the plastid translation machinery (Williams and Barkan 2003), while the defect leading to loss of ribosomes in *iojap* and *albostrians* are unknown at present. It has been demonstrated that temperature stress can

inactivate plastid ribosomes in rye, wheat, barley and oat (Feierabend and Mikus 1977; Feierabend and Schrader-Reichhardt 1976). Finally treatment of seeds of brassica species with the antibiotic spectinomycin (Zubko and Day 1998) or seeds of barley and maize with spectromycin (Zubko and Day 2002) followed by propagation of bleached seedlings in the absence of antibiotics results in stable albino plants lacking plastid ribosomes. Thus it is now well documented that various physical stresses and in particular treatment with aminoglycoside antibiotics can inactivate the ribosomes in a major part of the cells of ordinary seed derived embryos of several cereals (Zubko and Day 2002). Upon further growth and cell division, cells are formed with only ribosome deficient plastids, which cannot recover because translation of genes for central functions like the plastid encoded RNA polymerase is prevented.

While the hypothesis of ribosome deficient plastids seems to be the best explanation for the albino phenotype of many plants regenerated from cereal microspore cultures it does not clearly indicate the reason for the frequent formation of such plants particularly in the cereal species. Some further hints about the nature of the phenomenon may be obtained from genetics of the tendency to form green or albino plantlets. Throughout the history of cereal anther and microspore culture, differences between plant lines in their ability to produce high frequencies of green versus albino plants in the cultures have been noted (Andersen et al. 1987; Holme et al. 1999; Knudsen et al. 1989). Such genotype differences are, however, not qualitative in nature because of both sampling error with limited plants regenerated per line in most experiments as well as considerable unknown environmental effects on the donor plants. These genetic differences between plant cultivars may at first be expected to be inherited by genes in the plastids, but studies of green plant formation from crosses in wheat and barley (Larsen et al. 1991; Tuvesson et al. 1989; Zhou and Konzak 1992) have mostly shown no reciprocal effects, indicating that the trait is controlled by chromosomally inherited genes.

A large number of quantitative trait locus (QTL) studies of the ability to form high frequencies of green plants from anther culture, particularly from wheat, barley and rice have identified many different genes affecting the trait. However, there are indications that in general genes on wheat homoeologous group 2 and 5 chromosomes may play a role. Torp et al. (2001) identified three QTLs for green plant percentage on wheat chromosomes 2AL, 2BL and 5BL in the Ciano × Walter doubled haploid (DH) mapping population as well as one QTL on chromosome 5BL in a second DH population Ciano × Benoist. The QTL on chromosome 2BL, which was the most important, were subsequently confirmed in a new F_2 population developed from two DH lines of the original mapping population (Torp et al. 2004). In Barley, Chen et al. (2007) identified QTLs for percentage and number of green plants on chromosome 5HL, while QTLs for number of albino and green plants respectively were identified on chromosome 2H and 6H. In rice, He et al. (1998) identified major QTLs for green and/or albino plant differentiation frequency on rice chromosomes 1 and 9, homoeologous to wheat group 3 and 5 chromosomes, respectively. Thus in spite of the relative complexity of genes affecting the trait there are indications of major genes and common mechanisms of action across the cereal species.

Genes affecting the green plant regeneration frequency from microspores may be expected to be selected for during the *in vitro* culture. This would mean that green chromosome doubled haploids derived from F_1 hybrids by such cultures should have an increased frequency of the green plant promoting allele. However, several studies of segregation of such genes using linked markers have indicated the existence of both genes where the green type allele is selected during the culture as well as genetic loci where green and albino type alleles are equally represented among offspring from the microspores (Chen et al. 2007; He et al. 1998; Manninen 2000; Torp et al. 2001; 2004). These results may indicate different modes of action of the genes affecting green plant formation. Genes selected during the culture probably act locally in each haploid structure in a gametophytic mode so that haploid structures with the green promoting allele have a higher chance of regenerating green plants than structures with the albino promoting allele. Mechanism of action of such genes could be mediated through large gene products or structural differences in ribosomes or else where, which will not be exchanged between cells. In contrast genes not selected during the microspore culture may exert their effects through small molecules which can diffuse and be exchanged between cells, so that haploid structures with the green type allele do not have a higher chance of forming green plantlets than structures with the albino promoting allele.

We try to link the hypothesis of ribosome deficient plastids with the genetic information about genes affecting green plant formation frequencies. This is done through the study of the major gene affecting the trait on wheat chromosome 2BL. There is no or little selection between the alleles of this gene in anther cultures from F_1 hybrids between high and low responding parents (Torp et al. 2001, 2004) indicating action through a low molecular weight compound. Furthermore the green plant promoting allele is highly recessive relative to the allele promoting albino plant regeneration. This dominance situation indicates a lack of function mutation in lines producing many green plants, which is further supported by the fact that such high responsive lines are generally rare.

A simple way to combine the genetics with the hypothesis of ribosome deficient plastids would be to assume that ordinary wheat lines with low frequency of green plant formation produce low molecular weight ribosome inactivating compounds before or during the *in vitro* culture, which will generally inactivate most plastid ribosomes. High responding lines then will have mutations in one or more genes in the synthetic pathway of such compounds so that many plastids escape with intact ribosomes. Plants are known to produce a multitude of such chemical compounds some of which are involved in defence against pathogens (D'Auria and Gershenzon 2005). Since some of these compounds are likely to play more general roles in plants in addition to their role in defence, it could be hypothesized that they may also be induced in microspores or in the anther wall upon stress treatment. Both barley and wheat are capable of producing highly potent antibiotic like compounds including hordatines (Burhenne et al. 2003; Jin et al. 2003; Stoessl 1967), which can inactivate ribosomes like streptomycin (Venis 1969). During the stressful *in vitro* culture these plants may simply be fighting their own plastids.

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