Analysis of Chinese Spring regenerants obtained from short- and long-term wheat somatic embryogenesis

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Summary

Somatic embryogenesis was initiated from ‘immature embryos’ on Murashige-Skoog (MS) medium plus 2 mg.l⁻¹ 2,4-dichlorophenoxyacetic acid, 2% sucrose and 0.6% agarose. Somatic embryos were isolated and regenerated into whole green plants on MS medium devoid of 2,4-D. These regenerants were previously demonstrated to differ in their mitochondrial DNA organization. In order to estimate their characteristics three progenies of short-term culture regenerants and three progenies of long-term culture regenerants were analyzed and compared to the parental line. These somaclones obtained from the wheat variety Chinese Spring were evaluated for variation of 13 agronomic and morphological quantitative characters in comparison to the parental line. Significant variation was observed for plant height, spike length, main tiller diameter, between the somaclones regenerated from long-term culture and their parent. Differences were observed to increase with the duration of culture, leading to a significant modification of the structure of the plants. Several changes occurred during the somatic tissue cultures, but to a lesser extent than has previously been described in the literature.

Introduction

The occurrence of genetic variation in tissue culture and regenerated plants has been widely reported for polyploid species. This so called ‘somaclonal’ or ‘gametoclonal’ variation was noticed in wheat plants derived from different explant sources. Sometimes, genetic variation preexists in the cells of the explant (Charmet et al., 1986; Snape et al., 1988; Breiman et al., 1989) and in some other instances presumed changes in the regenerated plants originate from cross-pollination or admixture (Metakovsky et al., 1987). Nevertheless, there is evidence of various types of genetic variation, suggesting that there is no single origin but a range of multiplicity of contributing causes.

Changes occurring at the cytological level were shown for all species (Lee & Phillips, 1988), including changes in chromosome number (Orton, 1980; Karp et al., 1987), and structure (Gould, 1982; MacCoy et al., 1982; Karp & Maddock, 1984; de Buyser et al., 1985). Such changes were associated with reduced fertility and altered genetic ratios in the progeny of self-fertilized polyploid plants. Mitotic recombination could also account for some of the variation (Armstrong et al., 1983; Lapitan et al., 1984). Genomic rearrangements involving a homologous recombination process during mitosis were recently described (Prem Das et al., 1990). However, not all the variation in tissue culture can be attributed to cytologically observable changes. Many are due to more subtle changes. The stress of tissue culture seems sufficient to induce:

- mutations, e.g. single base pair changes. This could explain why several somaclonal variants showed a mendelian inheritance (Brettell et al., 1986).
- modification in the copy number of repeated sequences (Lapitan et al., 1988; Grisvard et al., 1990; Brown et al., 1990; Kidwell & Osborn, 1993).
activation of transposable elements such as Ac, Spm and Mu in maize (Peschke et al., 1987; James & Stadler, 1989; Peschke & Phillips, 1991) and the tobacco retrotransposon Tto (Hirochika, 1993).

- chloroplastic DNA loss (Day & Ellis, 1984).
- mitochondrial DNA reorganizations (Gengenbach & Connelly, 1981; Kemble & Shepard, 1984; Rode et al., 1987; Li et al., 1988; Brears et al., 1989; Hartmann et al., 1989; Shirzadegan et al., 1989).
- changes in DNA methylation patterns (Grisvard, 1985; Brown, 1989; Müller et al., 1990). In maize, 'all changes represented a decrease in methylation' and such changes were stably inherited (Kaeppler & Phillips, 1993). A reduced methylation of cytosine residues is associated with activation of the transposable element Ac (Brettel & Dennis, 1991).

Given the range of processes involved, it is probable that a number of them are operating simultaneously. Collectively they lead to variation for traits under simple genetic control as well as for quantitatively inherited characters.

Plants regenerated after short- and long-term somatic embryogenesis from immature embryos were previously demonstrated to possess clear and well documented modifications at the mitochondrial DNA level (Hartmann et al., 1989). The objectives of the present experiments were to evaluate these regenerants and their progeny for thirteen qualitative and quantitative characters.

Material and methods

Somatic tissue culture. Seeds of the wheat (Triticum aestivum L.) variety Chinese Spring were obtained from Dr J.W. Snape (IPSR, Norwich). Plants were grown in pots in growth cabinets containing mixed incandescent lighting with an intensity of about 200 µEm⁻²s⁻¹. Photoperiod was 16 hours and the day/night temperatures were 20°C/16°C. Immature kernels 14 to 16 days after controlled self-pollination were surface sterilized by a 2 second rinse in 95% ethanol and 2 minutes washing in 2% calcium hypochlorite. Kernels were then put on a piece of sterile paper and young embryos ('immature embryos') were dissected out and placed on Murashige-Skoog (MS) basal medium (Murashige & Skoog, 1962) plus 2 mgl⁻¹ 2,4-dichlorophenoxyacetic acid, 2% sucrose and 0.6% agarose for somatic embryogenesis. Embryogenic cultures were fragmented and pieces subcultured at 2 month intervals. Such a process produce 95–98% embryogenic cultures after 2 months and most of them have been maintained as long as seven years in culture. SC₁ and SC₆ refer to the first subculture (4 months in vitro) and the sixth subculture (14 months in vitro) respectively.

Plant regeneration. At the end of the first and the sixth subcultures, embryogenic culture pieces including somatic embryos, were transferred onto MS medium devoid of 2,4-D for regeneration. The cultures were maintained under a 16 hour photoperiod, with a reduced amount of light: 20 µEm⁻²s⁻¹ at 28°C ± 2°C. Regeneration was observed two weeks after subculture. Plantlets were put in tubes, containing Miller's medium gelled with a mixture of agar and geltrite in order to promote rooting (Henry & De Buyser, 1990). Regenerated, well-rooted plantlets were transferred to pots for continued growth. After some weeks plantlets were transferred for one month to a vernalization room (7°C ± 1°C). After hardening, the plantlets were put in a growth cabinet having a 16 hour photoperiod (day/night temperature of 20°C/16°C) and a high level of light (200 µEm⁻²s⁻¹). Plantlets were grown to maturity in large pots and commercial nutrient solution. The regenerated plants are referred to as the R₀ generation using the notation of Hartmann et al. (1989). Seeds produced by controlled self-pollination from R₀ were referred to as the R₁ generation. R¹ and R⁶ were referred to plants regenerated after 1 and 6 subcultures (SC₁ or SC₆). Sets of sexual progenies (non tissue-cultured plants) were developed in a similar manner to the regenerants. All the plants (CS, R¹ and R⁶) were grown under the same greenhouse conditions for one generation before the experimentation took place.

Morphological analysis. Agronomic and morphological variations were evaluated by planting the seeds in natural Spring conditions. Seeds of 6 regenerant lines at the second generation selfing (R₂) and the control variety of cultivar Chinese Spring (CS) were sown in the field at 5 cm intraline and 20 cm interline spacing. In these experiments a randomized design incorporated each genotype (CS, R¹ and R⁶) as a treatment, with three parental lines (CS non tissue-culture derived plants) and R¹ and R⁶ lines each with 40 plants. One extra line of the control (CS) was sown at each side as borders and eliminated together with the 5 first and the 5 last plants of each line, in the results. Performance of the lines were measured on the basis of 13 characters using 30 plants from each replication. The plants were