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# Polysomaty in growing in vitro sugar-beet (*Beta vulgaris* L.) seedlings of different ploidy level

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**Abstract**

In higher plants, nuclear DNA endoreduplication often occurs during growth and differentiation, which causes polysomaty of their tissues. Recognition of the pattern of endoreduplication in particular tissues and organs can be helpful in understanding the biological significance of this process. Polysomaty in different organs of diploid, triploid and tetraploid sugar-beet dry seeds and in seedlings during their early development in tissue culture (starting from radicle protrusion up to expansion of the first pair of leaves) was studied using flow cytometry. Radicles/roots, hypocotyls, cotyledons, leaves and petioles were analysed. Endopolyploidy level was organ-specific and characteristic of the stage of seedling development. It differed in plants of different ploidy and was the highest in diploid seedlings and the lowest in tetraploid ones. No endoreduplication occurred in dry seeds, young cotyledons and leaves. The highest mean *C*-value per haploid genome was in the hypocotyl before the first leaves appeared. A relationship between the development programme of the sugar-beet seedlings of different ploidy and the endoreduplication pattern is discussed.


*Keywords:* Somatic polyploidy; Flow cytometry; Diploid; Triploid; Tetraploid; Seedling development

## 1. Introduction

During differentiation of plant tissues, cells of various ploidy levels occur in one and the same organ, which is called polysomaty [1]. Cell polyploidisation is mainly due to either endomitosis or endoreduplication, and it occurs in over 90% of angiosperms [2]. Despite many studies on endopolyploidy, it is still not clear why the cell enters the endocycle instead of continuing proliferation. However, the relationship between endopolyploidy, ageing and differentiation of cells has been well known for a long time [3]. It has been hypothesized that polysomaty has a functional significance, including the need to coordinate gene expression required for the interaction of nuclear and organelle genomes [4,5]. Correlations between the nuclear DNA content, cell size and nuclear volume have also been found [6,7]. Endoreduplication has been proposed as a mechanism to generate sufficient DNA amount in anticipation of a future large increase in tissue mass. Nagl [4] suggested that DNA endoreduplication represents an evolutionary strategy which substitutes for a lack of phylogenetic increase in the nuclear DNA.

In sugar beet, endoreduplication was observed in developing and germinating seeds [8–10]. During development of triploid seed, the nuclei contain DNA from 2*C* to 32*C* in maternal (ovule), endosperm and embryo tissues [9]. The highest polysomaty occurs 14–24 days after pollination. However, in mature seed, only the cells in $G_0/G_1$ and $G_2$ phase of the cell cycle are present. Endoreduplication reappears in sugar-beet seed during germination and early seedling growth on filter paper in darkness at 20 °C [8]. Butterfass [11,12] found a presence of endoreduplicated cells in leaves and roots of mature diploid (up to 16*C*) and tetraploid (up to 32*C*) sugar beet. In experiments of Barow and Meister [13] diploid sugar beet revealed a very high degree of endopolyploidisation, especially in organs such as cotyledon, lower leaf and its stalk. Akinerdem [14] also

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observed polysomaty in old leaves of diploid and triploid sugar beet; however, only one round of endoreduplication was detected.

Sugar beet is a unique crop whose European cultivars are mostly triploid hybrids obtained by crossing a male-sterile diploid mother plant with a tetraploid pollinator. Several studies performed many years ago suggest that such hybrids give a higher root/sugar yield than diploid ones [15–17]. This was not confirmed by breeders from the USA (e.g. [18]) where, at that time, mostly diploid hybrid cultivars (both parental components diploid) were growing. Studies on tetraploid hybrids showed not only a lack of any beneficial effect of such high polyploidy on the crop yield, but even a decrease in a root weight [19,20]. It was concluded that the reason was a considerable proportion of aneuploids (30–35%) in the tetraploid plant population. The background for a possible superiority of the sugar-beet triploids over diploids, however, is still not clear. Studies on the polysomaty patterns in plants of different ploidy may provide more knowledge on this phenomenon and therefore, can be valuable for breeders in creating high productive cultivars.

The present study aimed at comparing the level of endoreduplication in different organs of diploid, triploid and tetraploid sugar beet during seedling development in vitro, in order to explain different performances of plants of different ploidy. The endopolyploidy patterns in diploid and tetraploid plants of maize [21], tomato [22] and portulaca [23] were previously established using flow cytometry. There are, however, no reports comparing polysomaty on the three different ploidy levels of the same species. Our study also provides useful information for sugar beet in vitro culture, since polysomatic explants may modify the ploidy level of regenerants [24,25].

## 2. Material and methods

### 2.1. Plant material and in vitro culture

Seeds of two cultivars, Arthur (2*x*), Kujawska (3*x*) and of breeding line IHAR (4*x*) were surface sterilized for 20 min in 5% sodium hypochlorite and washed with sterile water. Sterilized seeds were placed on the Murashige and Skoog (MS; [26]) medium in 200 ml jars, 8–10 seeds in each. Seedlings were grown in a growth chamber under a 16 h photoperiod at 22 ± 2 °C.

### 2.2. Developmental stages

The following developmental stages were distinguished: dry seed (stage 0), seedling with a root of 0.5–1 cm length (stage 1), seedling with a root of 1.5–2 cm length (stage 2), seedling with folded cotyledons (stage 3), seedling with unfolded cotyledons (stage 4) and seedling with unfolded first pair of leaves (stage 5).

### 2.3. Flow cytometry

Various organs of seeds and seedlings were analysed by flow cytometry (Tables 1–3). True seed was removed from the pericarp and divided into two parts, radicle and shoot axis. Seedlings at specific developmental stages were

Table 1
Percentage of nuclei with different DNA contents in organs of dry seeds and developing seedlings of diploid sugar beet

| Developmental stage | Organ | Percentage of the nuclei with DNA content | | | | |
|---|---|---|---|---|---|---|
| | | 2*C* | 4*C* | 8*C* | 16*C* | 32*C* |
| 0 | Radicle | 86 | 14 | | | |
| | Shoot axis | 98 | 2 | | | |
| 1 | Root | 30 | 43 | 23 | 4 | |
| | Cotyledon | 86 | 14 | | | |
| 2 | Root | 17 | 39 | 37 | 7 | |
| | Hypocotyl | 41 | 46 | 13 | | |
| | Cotyledon | 86 | 14 | | | |
| 3 | Root | 17 | 37 | 33 | 11 | 2 |
| | Hypocotyl | 18 | 34 | 28 | 18 | 2 |
| | Cotyledon | 32 | 61 | 7 | | |
| 4 | Root | 19 | 36 | 33 | 10 | 2 |
| | Hypocotyl | 17 | 33 | 26 | 22 | 2 |
| | Cotyledon | 25 | 66 | 9 | | |
| 5 | Root | 22 | 44 | 28 | 5 | 1 |
| | Hypocotyl | 19 | 36 | 20 | 22 | 3 |
| | Cotyledon | 22 | 57 | 18 | 3 | |
| | Petiole | 49 | 43 | 8 | | |
| | Leaf | 81 | 19 | | | |

Table 2
Percentage of nuclei with different DNA contents in organs of dry seeds and developing seedlings of triploid sugar beet

| Developmental stage | Organ | Percentage of the nuclei with DNA content | | | |
|---|---|---|---|---|---|
| | | 3*C* | 6*C* | 12*C* | 24*C* |
| 0 | Radicle | 79 | 21 | | |
| | Shoot axis | 97 | 3 | | |
| 1 | Root | 25 | 47 | 25 | 3 |
| | Cotyledon | 87 | 13 | | |
| 2 | Root | 19 | 44 | 33 | 4 |
| | Hypocotyl | 38 | 51 | 11 | |
| | Cotyledon | 83 | 17 | | |
| 3 | Root | 22 | 39 | 32 | 7 |
| | Hypocotyl | 17 | 36 | 34 | 13 |
| | Cotyledon | 35 | 59 | 6 | |
| 4 | Root | 21 | 40 | 31 | 8 |
| | Hypocotyl | 17 | 33 | 34 | 16 |
| | Cotyledon | 30 | 63 | 7 | |
| 5 | Root | 24 | 46 | 26 | 4 |
| | Hypocotyl | 25 | 33 | 31 | 11 |
| | Cotyledon | 28 | 53 | 17 | 2 |
| | Petiole | 49 | 43 | 8 | |
| | Leaf | 86 | 14 | | |