nected with the Schwann cell by such thin cytoplasmic passages. What may be the functional significance of these cellular liaisons, is difficult to discern. A simple hypothesis is that satellite cells supply the Schwann cell with products by which the latter can subsist for longer periods, in front of the post-synaptic membrane, in a denervated muscle.

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Variations in DNA content

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Summary. DNA content in the active nuclei of various plant organs varies from organ to organ. The amount of DNA in the shoot tip is the least, as compared to that of other organs. The amplification of DNA in the differentiation of organs from the shoot meristem has been suggested.

The importance of DNA amplification in differentiated tissue has been claimed by various authors[1-3]. It has been suggested that in plants there is a mechanism of phylogenetic increase of nuclear DNA through duplication, repeats and generative polyploidy. In absence of such a mechanism, an ontogenetic increase of nuclear DNA, as a prerequisite for differentiation, is adopted by the plants through endomitotic division[3]. The chromosomal behaviour in differentiated tissue has been cited as an example of dynamic behaviour of chromosomes[4,5].

In course of quantitative studies by us on in situ nuclear DNA, even in meristematic cells of different organs, variation in content has been observed[6]. As the cytological analysis shows their clear diploid chromosome content, these variations could not be attributed to difference in chromosome number.

There are, however, records of differences in amount of DNA in different organs, but such differences have mostly been reported in vitro involving polyploidy and aneuploidy[7,8]. No systematic attempt towards quantitation of the amount of DNA from the actively dividing meristematic regions of the organs has been given. In view of the fact that variation in DNA content has been observed through in situ analysis, the present investigation was planned to make a systematic study of quantity of DNA of nuclei from different organs from various plant tissues. For an accurate analysis, quantitation through cytophotometry at in situ level from cytological smears, as well as from nuclei after extraction from tissue, has been preferred.

The work was confined to the following species of plants - *Pisum sativum, Pisum sativum* var. B-22, *Lens esculenta, Trigonella foenum-graecum, Nigella sativa, Hordeum vulgare* and *Aloe vera.*

Seeds were germinated in potted soil. When the roots were about 2 cm long, meristematic and differentiated zones (1 mm and 2-3 mm from the tips respectively) were cut out separately. For the isolation of nuclei, the material was kept in 5% citric acid for 1 h to soften the tissue. Acid was decanted and the material was washed with buffer solution (10 mM Tris-HCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.25 M sucrose). Then 1 drop of mercaptoethanol was added to the material and it was homogenated with mortar and pestle and then with glass homogenizer with the buffer solution. The homogenate was then run through 2 layers of nylon cloth to remove the debris and then layered over 40% glycerol and centrifuged for 1 h at 10,000 rpm. The supernatant was discarded, the residue was suspended in 1.5% citric acid + 0.25 M sucrose, layered over 1.4 M sucrose, and centrifuged at 5000 rpm for 25 min. The nuclear pellet thus obtained was suspended in 0.25 M sucrose.

For cytophotometry (isolated nuclei), materials were fixed overnight in glacial acetic acid-ethanol mixture (1:2), treated with 5 N hydrochloric acid at 12-14 °C for 50 min-2 h, rinsed in distilled water and treated with Schiff's reagent for 2 h. Then the nuclei were isolated following the method already mentioned. Slides were prepared from the supernatant and mounted with 0.1% glycerol and sealed.

Table 1. Amount of DNA/nucleus (in situ) ($\times 10^{-12}$ g $\pm$ SE)

Species	Root meristem in metaphase	Differentiated zone	Shoot meristem in metaphase
Pisum sativum	6.9 ± 1.12	9.1 ± 0.82	5.5 ± 1.37
Pisum sativum var. B-22	5.2 ± 0.91	8.0 ± 1.41	3.7 ± 1.16
Lens esculenta	4.4 ± 0.76	6.1 ± 1.0	3.3 ± 0.91
Trigonella foenumgraecum	4.7 ± 0.06	6.8 ± 0.06	4.1 ± 0.09
Nigella sativa	6.5 ± 0.96	8.6 ± 1.16	5.7 ± 1.34
Hordeum vulgare	5.7 ± 1.65	8.0 ± 2.11	3.9 ± 1.38
Aloe vera	4.2 ± 0.93	8.5 ± 1.39	4.0 ± 0.98

Table 2. Amount of DNA/nucleus (extracted) ($\times 10^{-12}$ g $\pm$ SE)

Species	Root meristem	Differentiated zone	Shoot meristem
Pisum sativum	6.1 ± 1.39	7.0 ± 0.98	4.2 ± 0.55
Pisum sativum var. B-22	4.4 ± 1.13	6.0 ± 0.92	3.7 ± 0.65
Lens esculenta	4.3 ± 0.79	4.5 ± 0.88	3.0 ± 0.52
Trigonella foenumgraecum	3.9 ± 0.12	6.5 ± 0.09	2.1 ± 0.06
Nigella sativa	6.0 ± 0.92	7.8 ± 1.89	3.6 ± 1.23
Hordeum vulgare	5.4 ± 1.11	6.8 ± 1.06	-
Aloe vera	3.7 ± 1.67	6.5 ± 1.74	-

Preparations of root meristems, differentiated zones and shoot meristems for cytophotometry (in situ nuclei) were made by fixing in glacial acetic acid - ethanol mixture (1:2), followed by hydrolysis at 60 °C in 1 N HCl for 10 min. After hydrolysis, materials were washed in distilled water, stained with Schiff's reagent for at least 30 min, and squashed in 45% glacial acetic acid.

For cytophotometric analysis, Reichert Zetopan with microphotometer was used. 2 wave-length method[12] was adopted and observations as obtained from 100 nuclei of each set are presented below.

The results clearly indicate that the content of DNA varies in different organs of an individual. This is significant, as no chromosome number variation is noted in the metaphase plates counted in the meristematic regions in any of the species. The constancy in chromosome number with DNA variability in meristematic region is possibly an indication of polynemy in metaphase present during organogenesis. A significant feature is that in all cases the shoot meristem has been noted to contain a less amount of DNA as compared to others. Unpublished results of the authors from this laboratory, show that, even while the DNA contents of other organs are measured, the shoot meristem shows the least amount. The low content of DNA in shoot meristem may be due to the fact that it represents the initial step during organogenesis. The differentiation of organs is initiated at this level. Therefore the basic amount of DNA necessary for maintaining genetic stability is present in this region wherefrom differential DNA amplification may play a role in initiation of organ differentiation.

A comparison of the data on in situ and extracted nuclei shows that, inspite of the similarity of relative values, there is an overall decrease in amount of DNA in nuclei analyzed after isolation. This is attributable to the limitation of extraction procedure which may result in leaching out of some amount of DNA. Moreover, the extraction technique results in isolation of nuclei representing a mixture of those lying at G_1, S and G_2 phases, which also contribute to their lowered DNA value.

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Effects of calcium and daminozide on ethylene production and softening of apple fruits

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Summary. Ethylene production of McIntosh apples was reduced by both a postharvest treatment with $CaCl_2$ and a daminozide treatment applied in the field. The $CaCl_2$ treatment was more effective than daminozide in reducing the rate of softening during storage at 1 °C.

Fruit softening and its relationship to respiration and C_2H_4 production are fascinating and unsolved physiological puzzles. As well, the control of softening and fruit ripening are important practical problems. Calcium treatments reduce the respiration rate of fruits of apple[2] and avocados[3], retard senescence in some fruits[2] and reduce the rate of ripening in avocados[3] and tomatoes[4]. Yet an effect of calcium upon C_2H_4 production has been reported infrequently. Postharvest infiltration treatments with calcium have been noted to reduce C_2H_4 production during ripening of avocados[3], and orchard sprays of calcium reduced internal C_2H_4 content of apples[5]. Daminozide also reduces the respiration rate and C_2H_4 production of apple fruits and affects fruit firmness[6]. This paper describes a preliminary investigation of the effects of postharvest treatments of McIntosh apples with $CaCl_2$ after field treatments with daminozide.

Materials and methods. McIntosh apple trees were treated 37 days before harvest with 3 kg/ha of daminozide (wettable powder, 85% active ingredient). Fruit were harvested on 2 September 1976 in the preclimacteric stage as indicated by the pattern of respiration (CO_2 production) and C_2H_4 production. Before being placed in storage at 1 °C, 10-kg samples were dipped for 1 min in aqueous solutions of 6% $CaCl_2$+0.3% Keltrol, 6% $CaCl_2$ and 0.3% Keltrol (w/v). Samples were left dry as a control. The $CaCl_2$ was of 77% flake type and Keltrol (Ke), which is xanthan gum used to thicken and stabilize water-based solutions, was supplied as an 80-mesh powder. There were 3 replicates for each treatment and calcium treatments were imposed on the daminozide treatments in a factorial arrangement. Ethylene and CO_2 production were measured on 2-kg samples with an automatic sampling, flow-through system[7]. Firmness measurements were performed on pared flesh using an Effegi tester with 1.1-cm tip (10 fruit, 2 measurements per fruit). Analyses of variance were by standard procedures with differences among means for $CaCl_2$ treatments assessed using Duncan's Multiple Range Test at $p=0.05$ and the differences between daminozide treatments by the F-test at $p=0.05$.

Results and discussion. In the following discussion it has been accepted that Ca^{2+} is the effective ion as others have shown[8]. It is also accepted that Ca^{2+} has been taken up into the apple fruit following the pattern described by Betts and Bramlage[9].

The $CaCl_2$+Ke treatments consistently reduced C_2H_4 production (table 1). Although the effect upon CO_2 production was generally similar the differences were not always significant at $p=0.05$ (data not shown). Even the individual treatments of Ke and $CaCl_2$ reduced C_2H_4 production in