percentage of KOS-ROV cells with associations was significantly higher (73.14%). These variations may indicate that chromosome associations involve a very delicate structural process, influenced by several factors, such as sample manipulations in different laboratories[16,18-20], subjective judgement of the observer[3], age and sex of the subject[20]. These parameters, however, would seem to be unlikely to affect the nonrandom participation of specific chromosomes in the association figures. The kind of tissue used may be a factor influencing both frequency and nonrandomness in chromosome associations.

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Kaurene biosynthesis in intact *Phaseolus coccineus* suspensors

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Summary. Intact suspensors from *Phaseolus coccineus* seeds incorporate 2-(^{14}C)-MVA into a number of radioactive compounds, among which kaurene was identified by GC-MS. This result confirms that the kaurene biosynthetic pathway previously shown for cell-free extracts is active in intact tissues as well.

Studies of GA biosynthesis in higher plants have been mainly performed in cell-free systems. The pathway from mevalonic acid (MVA) to GA_{12}-aldehyde was firstly demonstrated by Graebe[1] in *Cucurbita maxima* and has been ascertained also in *Marah macrocarpus*[2] and *Pisum sativum*[3]. Although this pathway has been repeatedly suggested to be active also in intact tissues, very little information is available as to the direct biosynthesis of gibberellins in intact higher plants. One of the few reports available deals with the incorporation of 2-(^{14}C)-MVA into kaurene by leaves of *Stevia rebaudiana*[4].

We have previously described the pathway from MVA to kaurene[5] and from kaurene to ent-7α-hydroxy-kauren-19-oic acid in a cell-free system of *Phaseolus coccineus* (Lamarck) suspensors[6].

The biosynthesis of gibberellins by the same extracts fed with ent-7α-hydroxy-(^{14}C)-kauren-19-oic acid, previously biosynthesized by cell-free extract of *Phaseolus coccineus* suspensors, was demonstrated recently[7].

To test whether the pathway shown by cell-free extracts is actually active also in intact tissues, we incubated 2-(^{14}C)-MVA with suspensors freshly taken from *Phaseolus coccineus* seeds of 5–8 mm length. 1000 suspensors were incubated with phosphate buffer (0.05 M, pH 7.5), $MgCl_2$ (2.5 µM), ATP (5 mM) and 2-(^{14}C)-MVA (22.500.000 dpm at a sp. act. of 22 µCi/µmole) in a final volume of 450 µl, and shaken for 2 h at 28 °C. The reaction was terminated by adding 1 ml acetone, and after boiling for 2 min the tissue was homogenized, adjusted to pH 3 and extracted 3 times with EtOAc. The extract was then reduced to a small volume and chromatographed on silica TLC plates using hexane for development.

Radioactivity scanning revealed the separation of 2 peaks removed from the origin. The 2 fractions were then scraped, eluted and investigated by analytical and preparative GLC. The less polar fraction showed only 1 radioactive peak which co-chromatographed with an authentic kaurene standard. The identity of this compound as (^{14}C)-kaurene was definitively assessed by GC-MS and its specific activity, calculated on analytical GLC base, was 2.9 µCi/µmole. The MS-spectrum of the biosynthesized sample corresponds well with the standard kaurene MS spectrum obtained under the same conditions. We were unable to determine the identity of the other compound, separated by hexane, possibly due to the low level of the pool present originally in the tissue, as well as the nature of polar compounds that did not move from the origin.

In conclusion the incorporation of 2-(^{14}C)-MVA into kaurene shows that at least the early pathway of gibberellin biosynthesis is active in the intact suspensor, at the same developmental stage previously used to demonstrate the synthesis of GAs in a cell-free system.

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