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Laticifer and Rubber Particle Ontogeny in *Taraxacum kok-saghyz* (Rubber Dandelion) Roots

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Laticifers are specialized tubular vessels found in over 20 plant families [4, 6]. In general, laticifers can be categorized as non-articulated or articulated based on their origin, development and overall anatomy [7, 6]. Non-articulated laticifers develop from single cells. During non-articulated laticifer development, the cells become multinucleate due to the nuclei repeatedly dividing in order to fill in the growing distal parts of the laticifers with both nuclei and cytoplasm as the plant grows. Articulate laticifers have a compound origin from a series of cells which are united by dissolution of intervening walls [5]. Among plant species, about 12,500 plants have been reported to produce latex [4], but only 2,500 of them are rubber producing [1].

Taraxacum kok-saghyz (TK) is being developed as a commercial crop in the US and Europe [2, 3], as an alternative to *Hevea brasiliensis*, the tree that produces industrial rubber. Understanding laticifer origin and rubber particle ontogeny in this species will be useful in manipulating rubber production and yield. Thus, the objectives of this study are to gain a histological understanding of TK laticifer development and the ontogeny of rubber particles by characterizing the morphology and development of rubber-producing cells at both the seedling stage and in mature TK plants. Different microscopy methods were used to obtain high image resolution, and detailed analysis of early laticifer development and rubber particle ontogeny.

TK seeds germinated in the peat based media, PRO-MIX® BX, and seedlings were harvested daily for 30 days from the first day of germination to observe laticifer development by a simple histological staining method. Seedlings were fixed with a formalin-propiono-ethanol solution immediately after harvesting. Seedlings were then sectioned and stained using a mixture of three different stains; oil blue NA, safranin O and Congo red, to differentiate the rubber, lignified tissue, as well as cellulose and cytoplasm, respectively. The stained seedlings were examined using confocal (LEICA DMI 6000) and light microscopy (LEICA DM IRB). Roots from 10 month old TK were stained using the same methods so that comparisons could be made between developing and mature laticifers. Rubber particle ontogeny was observed in seedlings aged 6, 8, 10 and 12 days, which were collected, fixed, dehydrated and resin-infiltrated before being sectioned, stained and viewed under a TEM microscope (Hitachi H-7500). The same methods were applied to mature TK plants for comparison.

The microscopic observations on the seedlings showed that during germination, the primary roots emerge from the achene base. The root continued to elongate and began producing lateral roots five days after germination. Rubber particles were produced before laticifers, as early as eight days after germination, beginning at the cotyledonary collar areas and were visualized as blue fluorescence detected by confocal microscopy. Laticifers were not visible at the early seedling stage and were first observed 14 days after germination in the cotyledonary collar area at the pericycle and outside of the primary phloem group (Fig.1). On day 16, the elongation and anastomosing process were visible in the

cortex area. As seedlings age, the differentiation of laticifers became obvious, with the formation of cell rows, and fully formed cells with perforated end walls, that lead to anastomose formation with nearby laticifers.

The rubber particles, which are spherical or ovoid, were not as easily identified in seedlings as they were in mature TK plants. However, these particles began to appear 10 days after germination. The mature plants exhibited similar rubber particle morphology to *H. brasiliensis* and *Parthenium argentatum* (guayule). In TK, and like *H. brasiliensis*, rubber particles were produced in the cytoplasm of laticifer cells (Fig.2). In addition, laticifer plastids, membranous organelles found in the cytoplasm of laticifer cells, produced some of the rubber particles while other rubber particles were formed independent of this plastid (Fig.3). Also, two distinct rubber particle morphologies are found in TK. Rubber particles developed in the cytoplasm (cytoplasmic rubber) are irregularly shaped prior to moving inside the vacuole. Once in the vacuole, the outer layer of the rubber particle becomes smooth (vacuolar rubber). In conclusion, the findings have provided us with useful information on laticifer and rubber particle ontogeny that we can use further in studying and explaining the *de novo* site of rubber particles as well as to predict rubber production in TK roots. [7]

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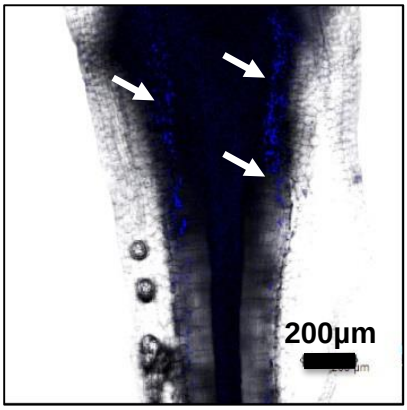

Figure1. Arrows indicates that the laticifers begin to appear first at the cotyledonary area 14 days after germination

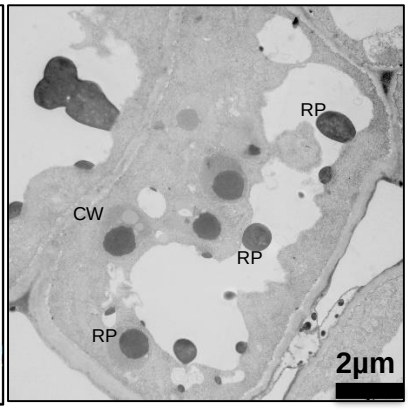

Figure 2. Laticifer cell in root of mature TK, Key: Rubber particles (RP); cell wall (CW)

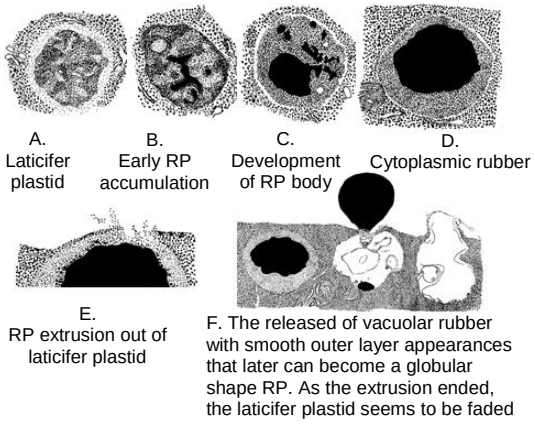

Figure 3. a-f. Stages of rubber particles (RP) produced from the laticifer plastid

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