

Notas Científicas

Latex production with depolymerizing compounds of actin cytoskeleton in rubber trees

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Abstract – The objective of this work was to assess stimulated latex flow from rubber trees (*Hevea brasiliensis*) with saturated macrolide (latrunculin A), 1, 5, and 10% potassium iodide in 2% methylcellulose compared with 0.3% ethylene in 2% methylcellulose (check) and 2% methylcellulose (blank). Latex output and contents of pure rubber, total solid, sucrose, inorganic phosphorus, thiol, and Mg^{2+} were measured. The treatments containing 1% KI or saturated macrolide increased latex yields compared to the blank with 2% methylcellulose alone. The 1% KI or saturated macrolide treatments were equal to that of 0.3% ethylene check treatment. However, 5 and 10% KI were harmful to bark of rubber trees, even caused prolonged tapping panel dryness.

Index terms: ethylene, latex yields, macrolide, microfilaments, physiological parameters, potassium iodide.

Produção de látex estimulada pela aplicação de compostos despolimerizantes de citoesqueleto de actina em seringueiras

Resumo – O objetivo deste trabalho foi avaliar a produção de látex em seringueiras (*Hevea brasiliensis*) estimulada pela aplicação de macrolídeo saturado (latrunculina A), iodeto de potássio a 1, 5 e 10%, e etileno a 0,3%, preparados em solução de metilcelulose a 0,2%. O tratamento testemunha consistiu da aplicação de solução pura de metilcelulose a 2%. Foram medidos a produção de látex e o conteúdo de borracha pura, sólidos totais, sacarose, fósforo inorgânico, tiol e Mg^{2+} . A aplicação de KI a 1% ou de macrolídeo saturado aumentou a produção de látex em comparação com o tratamento testemunha. Os tratamentos com KI a 1% e macrolídeo saturado foram iguais ao tratamento com etileno a 0,3%. No entanto, os tratamentos com KI a 5 e 10% foram prejudiciais à casca da seringueira, causando até mesmo o secamento do painel-de-sangria.

Termos para indexação: etileno, produção de látex, macrolídeos, microfilamentos, parâmetros fisiológicos, iodeto de potássio.

Plant cells respond to a variety of extrinsic and intrinsic stimuli with rapid and dramatic rearrangements of their cytoplasm (Nagai, 1993; Cyr & Palevitz, 1995). These changes are often mediated by dynamic cytoskeleton, the network of microfilaments, microtubules as well as their associated proteins (Fowler & Quatrano, 1997; Kropf et al., 1998). Most traditional studies of actin in a specific cellular response have been focused on cytoskeletal inhibitors including stabilizing agents (cytochalasins, phalloidin and jasplakinolide) (Sawitsky et al., 1999). Depolymerizing compounds or disrupters of actin polymerization (macrolide and potassium iodide – KI) are also involved (La Claire, 1989; Shimmen et al., 1995; Sawitsky, et al., 1999; Spector et al., 1999).

Natural rubber from rubber trees is strategically and widely used in military and civil industries. Today natural rubber can not meet market demands. Current research on rubber physiology is concerned about how to increase natural rubber yield without harm to the rubber trees (Luo & Deng, 2006). Ethylene can increase latex output of rubber trees at low concentration, but can cause some harm to the bark of rubber trees, even tapping panel dryness in the long run (Luo & Deng, 2006). Therefore, it would be helpful to develop a substitute for ethylene to increase natural rubber yields without side-effects.

The objective of this work was to assess the stimulation of latex production in rubber trees with different microfilaments depolymerizing compounds.

The experiment was accomplished at experimental farm of Chinese Academy of Tropical Agricultural Sciences in May, 2002. Thirty RY-7-33-97 rubber trees 55 cm in circumference in the same area were cut for the first time in 2002 and used for experimental samples. They were separated into six similar groups, providing each treatment with five replicates.

Methylcellulose was dissolved in distilled water at the concentration of 4%. After 4% methylcellulose solution was stirred and left standing 24 hours, different contents (1, 5 and 10%) of KI, saturated macrolide (latrunculin A) and 0.3% ethylene were added into above solution, which made the methylcellulose content drop to 2% (Table 1).

Before being treated by KI, macrolide or 0.3% ethylene, all the rubber trees for experiment were cut three times with s/2 and d/2 system in advance of treatment, but the latex was not gathered. From the fourth cut time forward (as the first cut time measured in the experiment), 5 mL latex samples were collected in ice baths each time, after rubber trees extruded about 20 drips of latex. Five grams of saturated macrolide, 0.3% ethylene, and the three different KI concentrations dissolved in methylcellulose were applied on the primal bark below 1 cm of cut panels and regenerated bark above cut panels along the wounds of the 20 rubber trees with a brush. Trees receiving 2% methylcellulose alone were the blank treatments. After 24 hours, rubber trees were tapped once every two days for ten days. Latex outputs at each cut time were recorded for ten days.

The latex from the five trees comprising a treatment at every cut time was collected in ice bath and regard as a mixed sample. The output of latex and six latex parameters (pure rubber, total solids, sucrose, inorganic phosphorus, thiol, and Mg^{2+}) were measured according to Eschbach et al. (1984) and Mo et al. (1999). The latex outputs of the treatments were analyzed with t test by SAS bio-software (Lin, 1998).

Table 1. Latex output of different treatments of depolymerizing compounds of microfilaments⁽¹⁾.

Treatment	Latex output (mL per cut time per tree)
2% methylcellulose (blank)	62.44b
0.3% ethylene + 2% methylcellulose (check)	85.34c
1% KI + 2% methylcellulose	88.70c
5% KI + 2% methylcellulose	49.96ab
10% KI + 2% methylcellulose	46.38a
Saturated macrolide + 2% methylcellulose	83.70c

⁽¹⁾Means followed by different letters are significantly different by t test, at 1% of probability.

The treatments 1% KI and saturated macrolide led to increase of latex output of rubber trees compared to the blank (Table 1) according to previous studies (Gao et al., 2001, 2003). The output was statistically equal to that of 0.3% ethylene check. The treatment with 1% KI produced more latex output than the 5 and 10% KI treatments without any side effects to rubber trees. However, La Claire (1989) reported that 0.6 M KI was the most effective depolymerizing compound of actin cytoskeleton.

Latex output increased with high content of KI (5 and 10%) compared to the blank at the first cut time. Then it decreased in the latter four cut times compared with the blank. There was tapping panel dryness on the bark at the same time. The cut panels and regenerated bark mildewed after a week treated by 5 or 10% KI. Moreover, the color of cortex turn into purplish-brown from sandy beige, along with excessive dehydration and kraurosis symptoms. These harmful symptoms were more serious on the younger rubber trees than on the older trees when they were treated with 5 or 10% KI.

Pure rubber content of latex from all treatments increased more or less (Figure 1 A). The largest content of pure rubber latex was from rubber trees stimulated by 10% KI while those from the rubber trees stimulated by 1% KI, saturated macrolide, 0.3% ethylene check and blank were similar. There were increasing total solid content of latex from rubber trees treated by 5 and 10% KI (Figure 1 B). Moreover, 0.3% ethylene, 1% KI and saturated macrolide treatments had similar trends of total solid content of latex, which increased from the first cut time to the third but decreased from the third to the fifth. The blank had no distinct changes in solids during the course of tapping. The content of pure rubber and content of total solid are the most important parameters to assess latex quality. As change trends of both parameters of latex stimulated by 1% KI, saturated macrolide, 0.3% ethylene control were similar, the quality of latex from those treatments was similar.

The content of Mg^{2+} in latex from rubber trees treated with saturated macrolide or 1% KI increased slightly (Figure 1 C). The content of Mg^{2+} in latex from those treated by 10% KI or 0.3% ethylene decreased from the first cut time to the fourth but increased from the fourth to the fifth. Magnesium ion content of latex from those rubber trees treated by 5% KI decreased during the course of tapping. Mg^{2+} content of latex from those rubber trees treated by 2% methylcellulose had no distinct changes during the course of tapping. The content

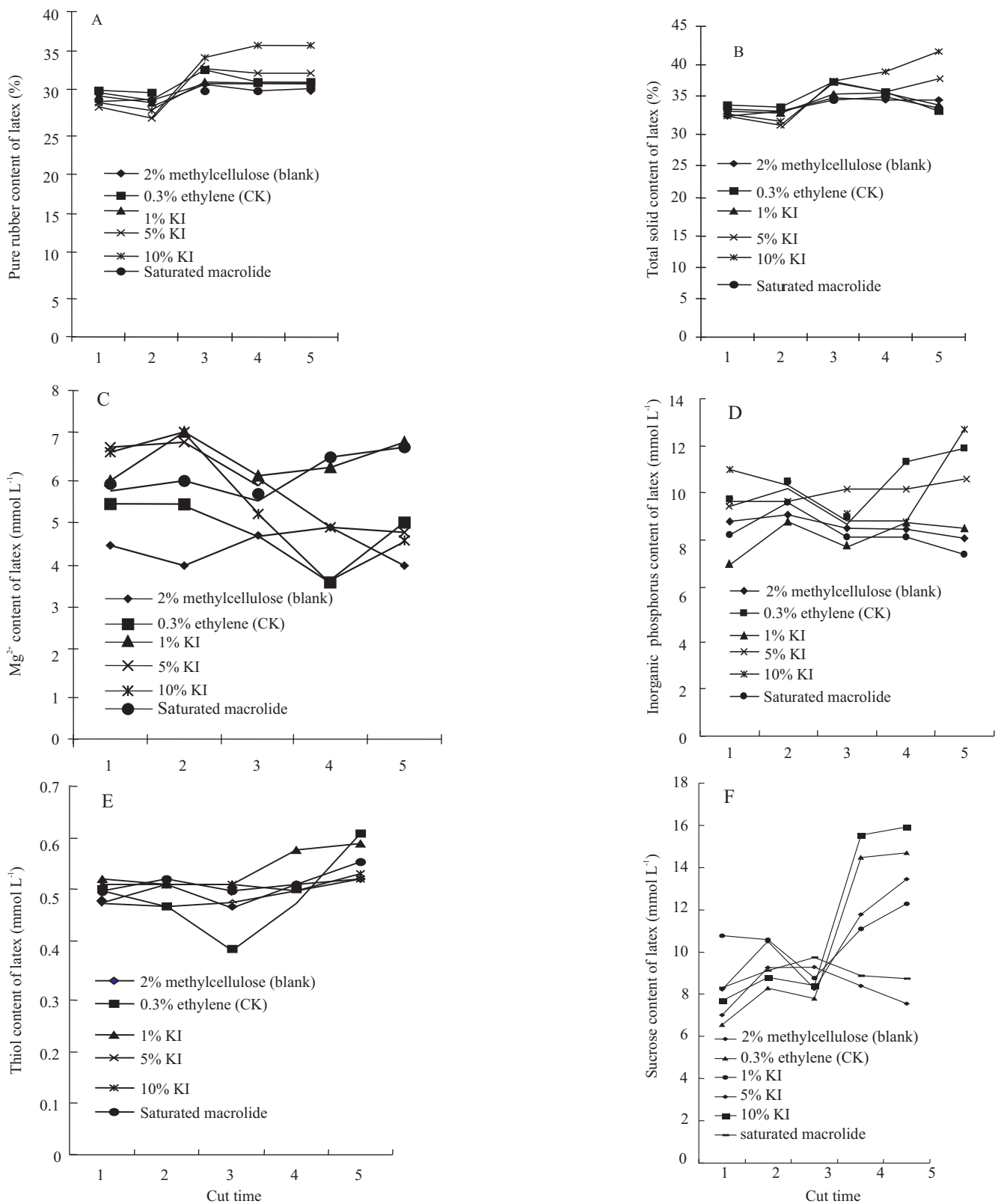


Figure 1. Changing trends in the content of pure rubber, total solids, Mg²⁺, inorganic phosphorus, thiol, and sucrose of latex from different microfilament depolymerizing compounds.

of inorganic phosphorus in the latex from rubber trees treated with 10% KI or 0.3% ethylene decreased lightly from the first cut time to the third and increased rapidly from the third to the fifth (Figure 1 D). The content of inorganic phosphorus in latex from rubber trees treated by 5% KI or 2% methylcellulose changed little during the course of tapping. Inorganic phosphorus content of latex from rubber trees treated by saturated 1% KI or macrolide had similar trends.

The content of thiol in latex from rubber trees treated by 0.3% ethylene decreased from the first cut time to the third cut time and increased quickly from the third to the fifth (Figure 1 E). Content of thiol in latex from other treatments increased slightly during the course of tapping. The sucrose content of latex from rubber trees treated by 10% KI, 0.3% ethylene, 5 and 1% KI rose slightly in the former three cut times and increased in the latter two (Figure 1 F). The content of sucrose in latex from those samples treated by 10% KI and 0.3% ethylene rose more quickly than that of latex from rubber trees treated by 5% KI. There was no change in latex sucrose content from rubber trees stimulated by saturated macrolide or 2% methylcellulose.

Magnesium ion, inorganic phosphorus, thiol, and sucrose content of the latex samples treated with 1% KI or saturated macrolide were different from 0.3% ethylene check treatment, which suggested that the mechanism of 1% KI or saturated macrolide treatment increase in latex output might be different from that of 0.3% ethylene. As depolymerizing compounds of actin cytoskeleton, 1% KI or saturated macrolide might lead to formation of protein-network and blocking of laticifers wound to stop latex overflow. As a result, the latex flow time was prolonged and latex yield increased (Gao et al., 2001, 2003).

Hao et al. (1996, 2004) found there was a compact protein-network composed mainly from lutoids, the vesicles of vacuo-lysosomes, formed gradually within and over the ends of the severed vessels during the course of latex flow. They assumed that it might be the results of signal transduction cascades converging on cytoskeletal proteins and redistributing cytoskeleton. Protein-network with rubber particles aggregations formed within and over laticifer wounds during latex flow might be regarded as a phenomenon which laticifer cells cytoplasm aggregated with actin cytoskeleton as framework in the wounds. Gao et al. (2003) showed that actin cytoskeleton played an important role in the formation of protein network. As framework, actin microfilaments captured rubber particle aggregations and

some kinds of defense proteins, such as chiniase and β -1, 3-glucanase within and over the laticifer cell wounds. Then the protein-network, as a biochemical barrier to prevent harmful pathogens from invading the laticifer wounds, might prevent components of cells from excessive overflow during the course of latex flow (Hao et al., 1996, 2004; Gao et al., 2003).

The application of 1% KI or saturated macrolide as depolymerizing compounds of actin microfilaments can increase latex yields. However, high concentration of KI (5 and 10%) might be potential harmful for rubber trees latex exploration.

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