Lights, Cold and Action! of ET as Abiotic Stresses Influencing Rubber Production in *Taraxacum kok-saghyz* Roots

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ABSTRACT

Rubber yield can be manipulated, and often increased, by environmental and hormonal methods as proven in *Hevea brasiliensis*, the world's main rubber producing plant. However, similar efforts can be implemented on an alternative rubber plant, *Taraxacum kok-sagyz* (TK), where even slight gains in yield are beneficial towards its commercialization. This study aims to identify methods of applying abiotic stress on TK to improve rubber yield. Different physiological and environmental factors will be tested on the plants, including light quality; cold induction using a hydroponics system and manipulation of the hormone ethylene via the use of ethephon. The treatment effects were monitored by analyte quantification using Accelerated Solvent Extraction (ASE) as well as by microscopy. The findings indicate that rubber increased with these different physiological and environmental approaches. Supplemental lights doubled total rubber with 14.2±4.3 mg/root whereby the plants in the natural light treatment only yielded 5.7±1.4 mg/root. After 50 days, cold treated plants had significantly higher rubber per root with 325.5±50.0 mg/root in comparison to the control (123.3±20.1 mg/root) in the hydroponics system and 1% ethephon solution at ambient temperature increased rubber concentration significantly (P<0.05) after nine days, with 81.9±4.3mg/g in comparison to the distilled water control level of 50.7±4.4mg/g. Furthermore, a histological study shows cellular changes in the cytoplasm and a production of morphologically distinct rubber particles when under the stress conditions. Overall, these studies are providing valuable information on abiotic stresses on TK rubber biosynthesis which can be adapted into the agronomic practices of this crop.

INTRODUCTION

Taraxacum kok-sagyz (TK) is currently being developed as a commercial crop in the US and Europe (Buranov and Elmuradov, 2010; Cornish et al., 2013) and is an alternative for *Hevea*, the tree that produce rubber industrially. As TK is still at its early stage of plant domestication and commercialization, methods are needed to maximize rubber production especially through its horticultural practices. Recently a series of studies were conducted with the objectives to identify possible environmental or chemical means as an abiotic stresses that can be used to improve rubber yield in TK and can be implemented as horticultural/agronomic and post-harvest practices. In these studies, supplemental light, cold induction and ethylene application via the use of ethephon (ET) were evaluated as an effort to provide information of abiotic stress effects on TK rubber biosynthesis.

MATERIALS AND METHODS

The supplemental light study involves the use of LED lights on hydroponically-grown TK. Clonal planting material was used and grown using nutrient film technique (NFT) under 16-hour exposure of LED lights and were compared with natural light exposure in summer 2015. The plants were harvested after 2 and 3 months with the photosynthetic rates were monitored and recorded using LICOR (Li-6400) equipment. Meanwhile for the cold induction study, clonal planting material from a single plant was grown with NFT systems as well. After 4 months, cold temperature (10 ± 2°C) was introduced by using chillers. The plants were harvested after 25 and 50 days. In ET experiment, one-year old TK plants were harvested and the roots were soaked in four different treatments: distilled water (control) or 1% ET at either ambient temperature or 4°C. After 9 days, the roots were harvested for analysis. Analytes quantification was used in all three experiments by quantifying inulin, resin and rubber content with accelerated solvent extraction (ASE) (Pearson *et al*., 2013). Latex quantification (LQ) (Cornish et al., 1999) and PENRA III method which used enzymatic process were carried out as well. For microscopy study, samples were fixed, dehydrated and resin-infiltrated before being sectioned, stained and viewed under a TEM microscope (Hitachi H-7500).

RESULTS AND DISCUSSION

SUPPLEMENTAL LIGHT

[Rubber] was influenced by the introduction of the supplemental lights and the changes can be associate with higher photosynthetic rate

Table 1a: Summary of two-way ANOVA for root weight and the analytes with treatment and month as the source of variation

Source of variation	Root weight	Rubber	Resin	Inulin
Treatment	0.06ns	5.08*	0.01ns	0.49ns
Month	7.38*	10.70**	9.78*	3.71ns

LSD, ns, p≥ 0.05; *, p< 0.05; **, p< 0.01

Table 1b: Summary of four-way ANOVA on selected physiological parameters

Source of variation	PAR	A	g_s	WUE
Treatment	7.12**	42.81***	1.14ns	0.98ns
Month	43.17***	591.21***	1.18ns	47.74***
Weather	5.05*	8.87**	1.82ns	10.27**
Hour	8.30***	9.54***	0.2ns	4.23*

LSD, ns, p≥ 0.05; *, p< 0.05; **, p< 0.01, ***, p< 0.001 PAR; photosynthetically active radiation, A; photosynthetic rate, gs; stomatal conductance, WUE; water use efficiency

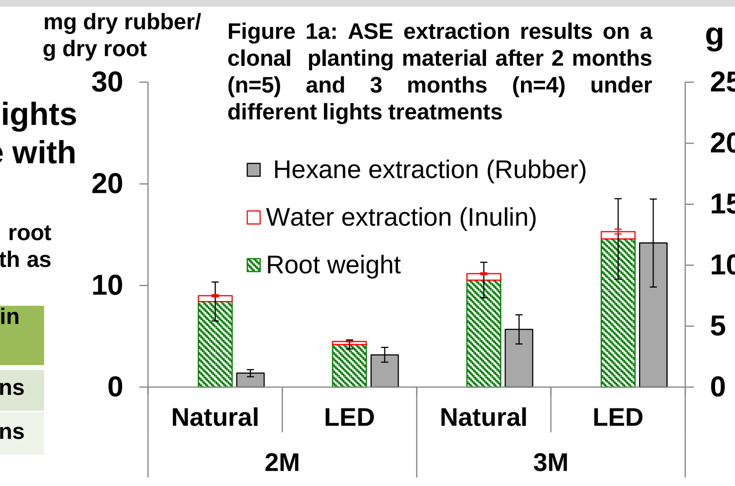


Figure 1a: ASE extraction results on a clonal planting material after 2 months (n=5) and 3 months (n=4) under different lights treatments

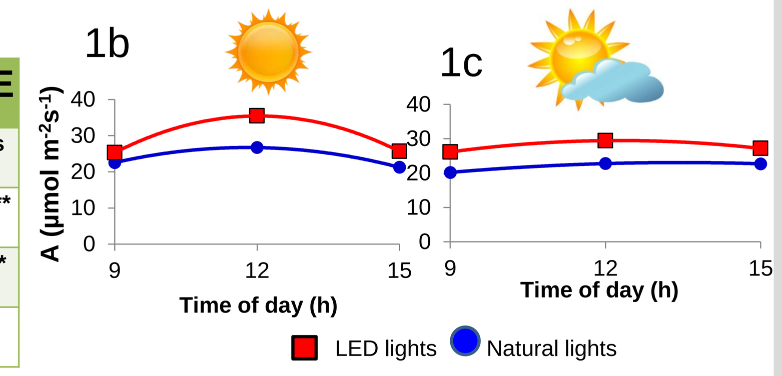


Figure 1b: Photosynthetic rate (A) measured during sunny (1b) and cloudy (1c) day at 9AM, 12PM and 3 PM indicates that higher "A" was recorded during the use of supplemental lights

COLD INDUCTION

Inulin was observed to be affected by cold meanwhile latex/root increases after 50 days of cold induction, indicating that a hydroponics system would be suitable for latex production

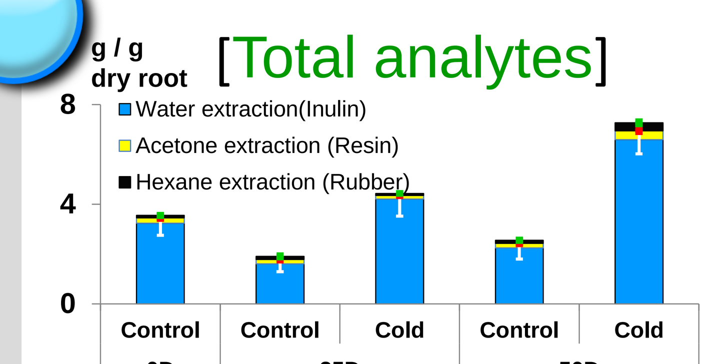


Figure 2a: ASE extraction results with n=12 plants, except for control plants, 25 days after the treatment begin (n= 6)

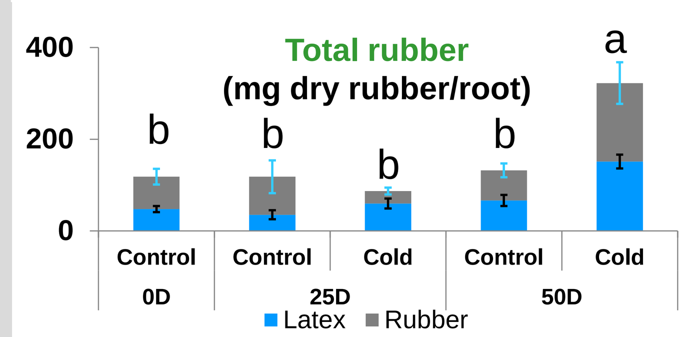
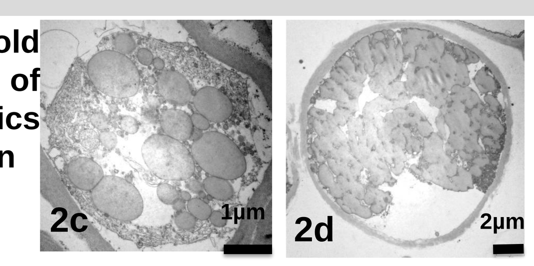


Figure 2b: Latex quantification (LQ) results. Means with the same letter(s) are not significantly different at p<0.05 (LSD test)

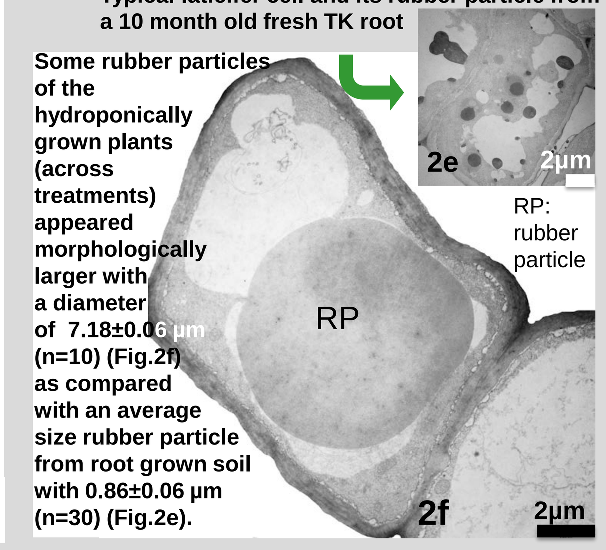


50 days after cold induction (Fig.2d) and of a control laticifer cell of same age under the NFT system (2c) shows different rubber particle accumulation pattern

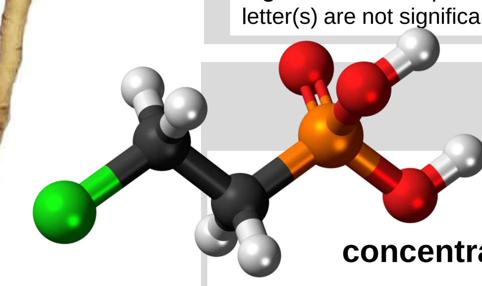
Rubber particle Ø

Typical laticifer cell and its rubber particle from a 10 month old fresh TK root

Some rubber particles of the hydroponically grown plants (across treatments) appeared morphologically larger with a diameter of 7.18±0.06 µm (n=10) (Fig.2f) as compared with an average size rubber particle from root grown soil with 0.86±0.06 µm (n=30) (Fig.2e).



ETHEPHON



Ethephon increased rubber concentration in the treated roots over 9 days

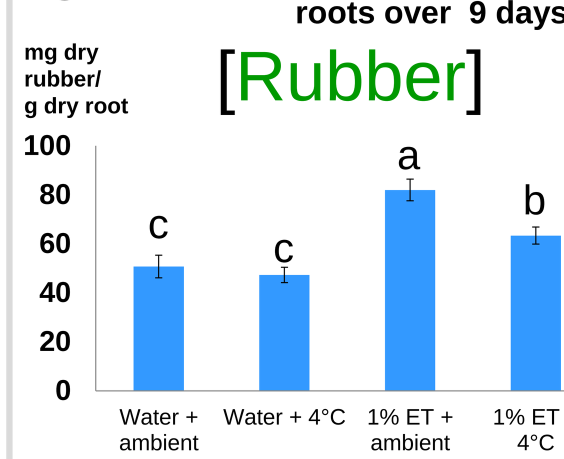
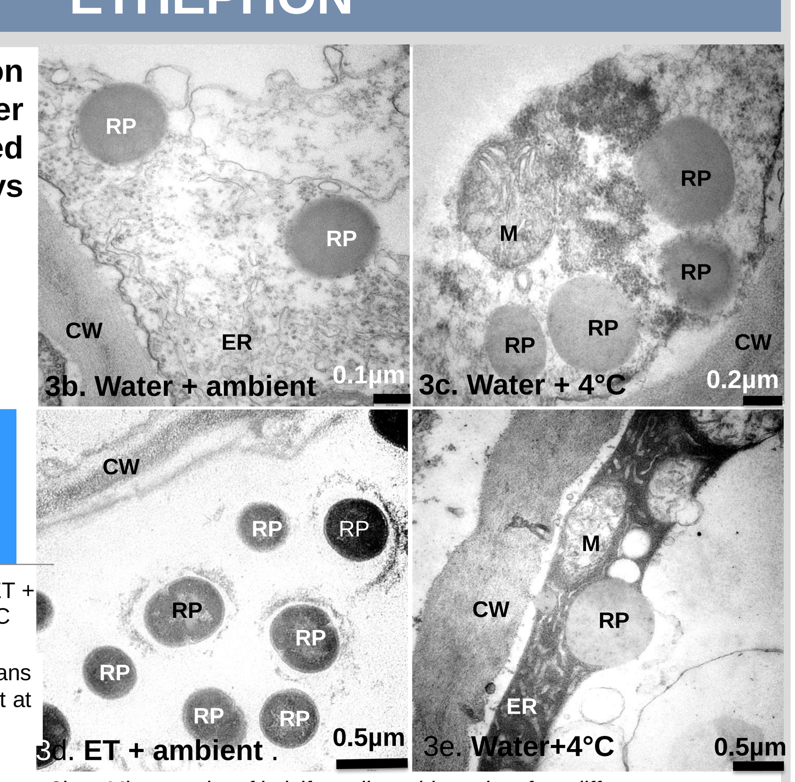


Figure 3a: ASE results of 30 plants/treatment. Means with the same letter(s) are not significantly different at p<0.05 (LSD test)



3b-e; Micrographs of laticifer cells subjected to four different treatments Key: Cell wall (CW);endoplasmic reticulum (ER); rubber particle (RP); mitochondria (M)

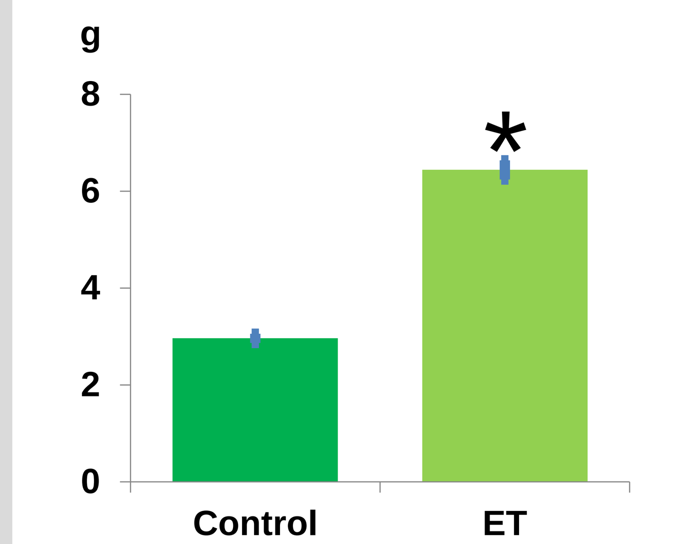
Water + ambient <Water+4°C< ET+4°C< ET+ ambient

Cytoplasmic senescence rate

Based on microscopy observations, we suggest that ET at ambient temperature stimulates the rate of cytoplasmic senescence and the cytoplasm breakdown products are channeled into rubber particle biosynthesis.

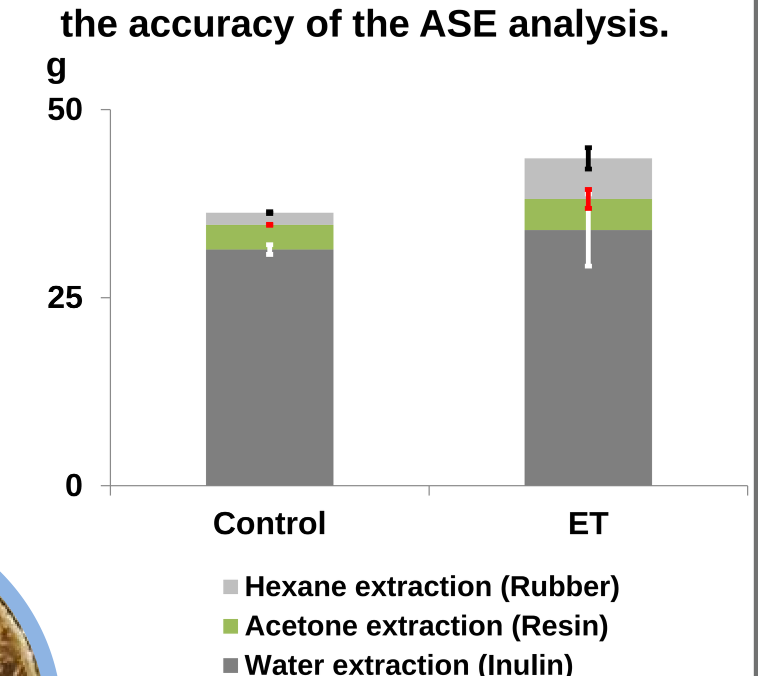
Manipulation of abiotic stress in TK rubber production: The case of ET

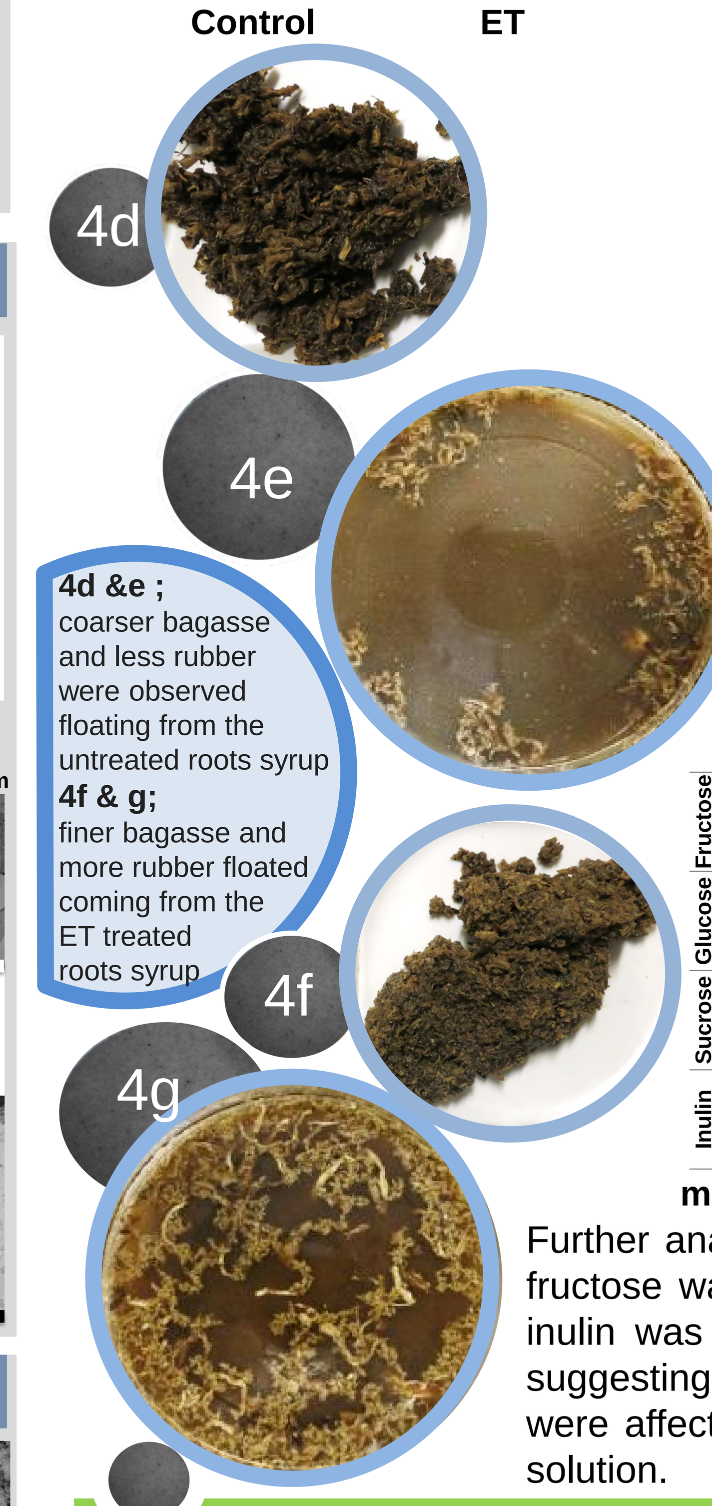
Figure 4a. g rubber/100 g dry weight roots obtained from PENRA method. The s.e's reflect the accuracy of the method with mean comparison using t-test



An attempt has been made to combine ET with rubber processing PENRA III method that uses enzymes to increase yield and purity in TK rubber. Initial result indicate that ET treated roots combined with PENRA III method obtained 113% more rubber in comparison to non-treated dried fresh roots that undergone the same processing method.

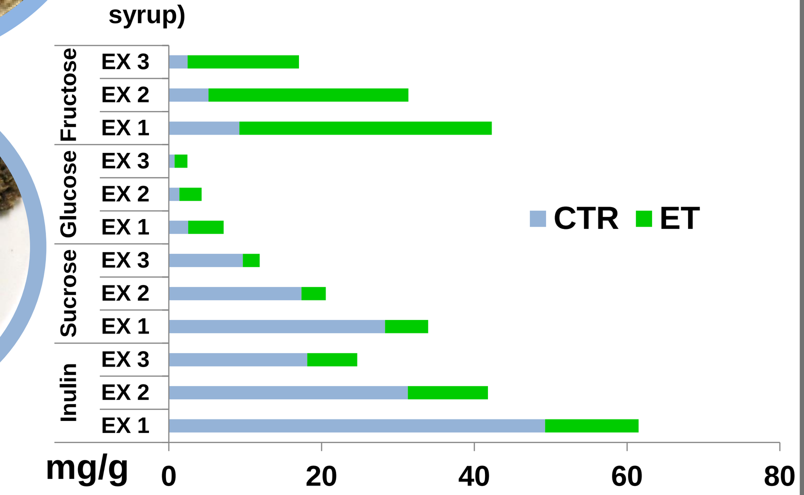
Figure 4b. g rubber/100 g dry weight roots obtained from the ASE extraction method. The s.e's reflect the accuracy of the ASE analysis.





4d &e ; coarser bagasse and less rubber were observed floating from the untreated roots syrup
4f & g; finer bagasse and more rubber floated coming from the ET treated roots syrup

Figure 4c. mg sugar/g root of different types obtained from the HPLC
(EX = extraction from different batches of boiled root syrup)



Further analysis on the sugar content revealed that fructose was high with ET treated roots meanwhile inulin was high from the un-treated roots. We are suggesting that carbohydrate translocation patterns were affected by stress induced from the acidic ET solution.

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