HIGH LIGHT AND OSMOTIC STRESS INDUCED FRAGMENTATION OF GENOMIC DNA IN *HEVEA BRASILIENSIS*

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Plant nuclear DNA is an inherently unstable molecule and can be damaged metabolically or by a number of stress factors like extreme temperatures, drought and pathogen attack. In the present study, excised leaf discs harvested from two *Hevea* clones, RRII 430 (relatively drought tolerant) and RRII 414 (relatively drought susceptible) were subjected to water deficit stress *in vitro* using PEG under low and high light conditions in a plant growth chamber and another set of leaf discs kept under sunlight in open field. The integrity of genomic DNA from the leaf discs subjected to the stress conditions indicated a fair degree of DNA fragmentation in drought susceptible clone under high light alone as well as in the combination of high light and PEG stresses. In the drought tolerant clone, DNA was comparatively intact with no visible signs of fragmentation. On the other hand, under very high light conditions in the open field, significant level of DNA fragmentation was observed in both the clones indicating that high light can inflict serious damages to DNA in both drought tolerant and susceptible clones.

Key words: DNA fragmentation, *Hevea brasiliensis*, High light intensity, Osmotic stress

The ideal agro-climate for natural rubber (*Hevea brasiliensis*) cultivation is a wet and warm humid tropical environment with plenty of sunshine. Due to non-availability of land in traditional rubber growing regions, its cultivation is being extended to non-traditional areas where stressful climatic conditions limit growth, development and productivity of *Hevea* (Jacob *et al*., 1999). Drought is probably the most important factor that limits natural rubber productivity in India. This is also the most important factor that restricts the expansion of its cultivation to newer areas in several rubber growing countries.

Under field conditions excess light also can be a source of stress. Plants are usually exposed to drought and high light conditions at the same time. High light intensity aggravates the harmful effects of drought stress (Chaves *et al*., 2002; Szechynska-Hebda and Karpinski, 2013). Excess light leads to over production of excited electrons far in excess of what is needed to reduce CO_2 through photosynthesis and this also will lead to production of reactive oxygen species (ROS) (Jacob and Karaba, 1998; Jacob and Lawler, 1993; Sharma *et al*., 2012). Plant nuclear DNA is an inherently unstable molecule and can be damaged metabolically

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or by a number of stress factors like extreme temperatures, drought and pathogen attack (Ryerson and Heath, 1996). DNA fragmentation has been observed in plants due to high light and drought stress (Danan and Gollois, 1998; Wituszynska and Karpinski, 2013). Over-production of ROS as byproducts of normal cellular metabolism or as a result of abiotic stress conditions leads to DNA damage in plant cells (Gill and Tuteja, 2010; Petrov *et al*., 2015).

Stress tolerant varieties develop several adaptive mechanisms to manage or escape the adverse impacts of environmental extremes. Understanding the mechanisms of drought tolerance in *Hevea* is central to any crop improvement research for evolving new clones with better tolerance to climate stress. In the present study, two *Hevea* clones with different levels of drought tolerance capacity were subjected to *in vitro* water deficit stress under low and high light conditions and the integrity of the genomic DNA was analysed to examine whether the tolerant clone maintained its DNA more intact than the susceptible clone under conditions of drought and high light stress.

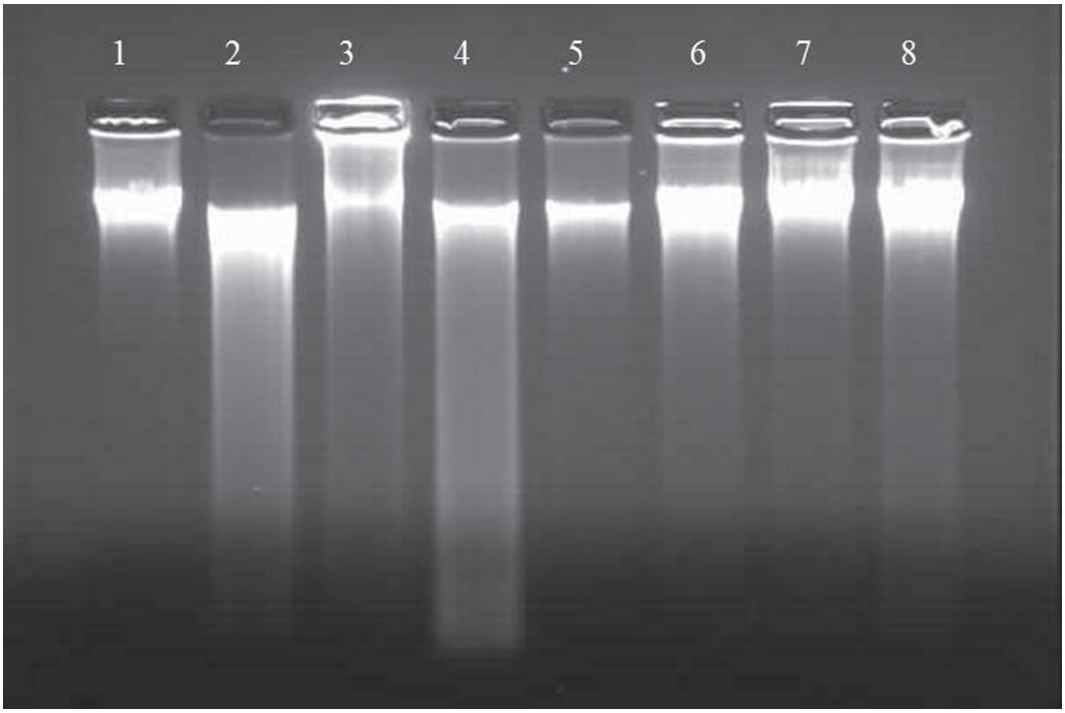

Fig. 1. Visualization of genomic DNA (2 µg) from *Hevea* leaf tissue on 1% agarose gel. The leaf discs were incubated in PEG 40% / distilled water at low light at room temperature and high light in the growth chamber.

Lane 1 - RRII 414 Low light (water)
Lane 2 - RRII 414 High light (water)
Lane 3 - RRII 414 Low light + 40% PEG
Lane 4 - RRII 414 High light + 40% PEG
Lane 5 - RRII 430 Low light (water)
Lane 6 - RRII 430 High light (water)
Lane 7 - RRII 430 Low light + 40% PEG
Lane 8 - RRII 430 High light + 40% PEG

Leaf discs of diameter 1.5 cm were prepared from physiologically mature leaves collected from two polybag grown *Hevea* clones, RRII 430 (relatively drought tolerant) and RRII 414 (relatively drought susceptible) belonging to the latest series of high yielding clones released by Rubber Research Institute of India. Leaf discs were incubated in petri dishes containing poly ethylene glycol (PEG 40 %). One set was kept at dark at room temperature (25°C) and another set was exposed to high light in a growth chamber for three hours. The following conditions were maintained in the growth chamber; light intensity: 1000 µmol m^{-2} s^{-1}, RH: 60 per cent and temperature: 30°C. One more set of leaf discs from both the clones was incubated in distilled water and kept for three hours in the open field with sun light intensity ranging from 1600 to 1800 µmol m^{-2} s^{-1} and temperature 34°C to 37°C.

At the end of the incubation period, the leaf discs were washed thoroughly with distilled water and genomic DNA was extracted following the method of Porebski *et al*. (1997) with modification (Thomas *et al*., 2001). The concentration of DNA in each sample was determined with a Nanodrop spectrophotometer (USA).

Five µL of loading buffer (0.25% bromophenol blue, 30% glycerol in TE buffer, pH-8.0) were added to 15 µL of the DNA (2 µg) and the samples were loaded on to a 1 per cent agarose gel prepared in 1X TAE buffer (Tris - Acetate - EDTA). Electrophoresis was carried out at 50 volts until the bromophenol blue dye front migrated to the bottom of the gel. Staining was carried out with 0.5 µg mL^{-1} ethidium bromide and the gel was photographed under UV light.

Water deficit stress and high light stress lead to severe fragmentation of DNA in both clones. In the drought tolerant clone (RRII 430), DNA fragmentation due to water deficit or high light was comparatively less than in the drought susceptible clone (RRII 414). Under very high light (open sunlight) conditions, DNA extracted from both the clones were more or less similarly damaged (Fig. 2), indicating that high light can inflict serious damages to DNA in both drought tolerant and susceptible clones. Light with intensity higher than 1000 µmol photons m^{-2} s^{-1} can be very damaging to

Fig. 2. Visualization of genomic DNA (2 µg) from *Hevea* leaf tissue on 1% agarose gel. The leaf discs were incubated in distilled water for 3 hours under open sunlight with light intensity in the range of 1600 to 1800 µmol m^{-2} s^{-1}.
Lane 1 - RRII 414 Low light
Lane 2 - RRII 414 High light
Lane 3 - RRII 430 Low light
Lane 4 - RRII 430 High light

plants (Szechynska-Hebda and Karpinski, 2013). Both under moderately high light and water deficit stress (growth chamber condition), DNA fragmentation was observed in the drought susceptible clone, RRII 414 (Fig. 1).

As a response to stress caused by diverse environmental factors, higher plants have evolved adaptive mechanisms at the physiological, cellular and molecular levels. Despite the stable nature of plant genome, nuclear DNA is an inherently unstable molecule and can be damaged spontaneously, metabolically or by abiotic stress factors (Tuteja *et al*., 2009). Garces *et al.* (2001) observed mechanical stress elicited nitric oxide formation and DNA fragmentation in *Arabidopsis thaliana*. Ryerson and Heath (1996) observed cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic stress.

One of the reasons for DNA fragmentation under drought stress is the generation of ROS (Gill and Tuteja, 2010; Gill *et al*., 2015; Petrov *et al*., 2015). In the present study, DNA isolated from leaf discs was found more intact in the drought tolerant clone under moderately high light and PEG stress which may be due to better anti-oxidant scavenging system in this clone. Significantly higher levels of ascorbic acid (antioxidant) content and super oxide dismutase (SOD) activity were observed in RRII 430 (Thomas *et al.*, 2014). At very high light intensities, the clonal differences in DNA protection disappeared even in the absence of PEG stress. This shows the overwhelming effect of high light intensity to inflict damage to DNA even in a healthy cell. Thus high light may act as an aggravator of harmful effects of other abiotic stresses. Further studies are needed to elucidate the mechanism of DNA protection under stress in the tolerant clone and utilize this finding for identification of drought tolerant clones.

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