1988

CAN DIFFERENCE IN THE GENETICS BETWEEN THE ROOT STOCK AND SCION LEAD TO TAPPING PANEL DRYNESS SYNDROME ?

Sobhana, P., Molly Thomas, Krishnakumar, R., Saha,T., Sreena, A.S and James Jacob.

Rubber Research Institute of India, Kottayam, Kerala, India.

Abstract *In the present study we hypothesized that a greater genetic distance between rootstock and scion may interfere with the physiology of the scion, eventually leading to symptoms of delayed incompatibility culminating in a physiological disorder like tapping panel dryness syndrome (TPD) in Hevea brasiliensis. This was addressed by subjecting the bark tissues from rootstock and scion portions of healthy and fully TPD affected trees to isozyme and RAPD analyses. As expected the RAPD profiles indicated perfect genetic homogeneity between the scion tissues (genetic distance =0) because all the scion samples came from the same clone, GT1. The genetic distance among the rootstock tissues (which were grown from heterozygous seeds) ranged from 7-39%. The isozyme profile of the enzyme peroxidase showed variability among the genetically homozygous scion tissues as well as the heterozygous rootstock tissues, indicating an influence of rootstock on the scion. While the changes in the RAPD profiles between two trees could be attributed to true genetic differences, the same cannot be said with reference to enzyme polymorphism which is more related to gene expression. The initial indications from the present study are that the genetic distance between rootstock and scion tissues was higher in the TPD affected than healthy trees. The implications of this observation in various aspects of Plant Biology are discussed.*

Key words: *Hevea*; genetic; rootstock; scion; budgrafting; tapping panel dryness

INTRODUCTION

Bud grafting is the most popular means of propagation of *Hevea brasiliensis*. This ensures genetic homogeneity of the scion in this highly heterozygous species, but the rootstocks which are grown from cross-pollinated seeds are heterozygous in nature. The very large intraclonal variability that is noticed in growth and yield of budgrafted clones of *Hevea* is attributed to the genetic heterogeneity of the rootstocks (Yeang *et al*., 1995; Sobhana, 1998).

There are several reports showing the effects of the rootstocks on the physiology of the scion in *Hevea*. For example, the isozyme polymorphism that is found in the scion tissues has been attributed to the rootstocks (Krishnakumar *et al*., 1992). The rate of photosynthesis of young bud grafted plants was positively correlated with the rate of photosynthesis of the stock seedling just before bud grafting (Sobhana, 1998). Several other physiological functions of the scion were also found to affect by those of the root stocks (unpublished data). It has been shown in other tree crops such as apple, plum, peach, etc., that if the rootstock and scion are genetically divergent, there can be physiological problems occurring in the scion at later stages of the plant growth (Andrews and Marquez, 1993). This kind of delayed incompatibility is often expressed in very subtle forms and need not result in the failure of the bud union between

the scion and the rootstock which is considered as incompatibility from a more applied horticultural perspective.

In a bud grafted *Hevea* plantation of a given clone, there may be trees having similar or dissimilar genetic profiles between the rootstocks and the scions and the extent of similarity or otherwise could be different among individual trees and randomly distributed in the field. Can TPD be a manifestation of some kind of delayed physiological incompatibility between the root-stock and scion? We hypothesize that the greater the genetic distance between the root stock and the scion, the greater the possibility of the scion showing symptoms of TPD which is also randomly distributed in the field. As a first approach to test this hypothesis, we sampled the bark tissues from the rootstocks and scions of two healthy and three fully TPD affected trees and subjected them to isozyme and RAPD analyses. The trees belonging to the clone GT1 were 18 years old and have been under the S/2 d/2 tapping system. These trees were under regular monthly observation for more than one year before their TPD status was ascertained. Because of the fairly long period for which they had been tapped and that the trees were under close observation during tapping, the TPD status of the selected trees can be believed to be accurate with a high degree of confidence.

EXPERIMENTAL

Methods and Materials

Bark samples were collected from the rootstock and scion portions of the above trees. 500 mg of soft bark was crushed in liquid nitrogen and homogenised with 2ml of extraction buffer (0.1M Tris-HCl pH 8.0, 0.2% 2-mercapto ethanol, 0.001M EDTA, 0.01M $MgCl_2$) and 15% insoluble PVP. The homogenates were centrifuged at 20000 rpm for 20 minutes at 0°C. The clear supernatant was used for analysis of the isozyme. Phast Gel Electrophoresis System (Pharmacia, Sweden) was used for the horizontal isoelectric focusing (IEF) on ultra thin mini polyacrylamide gel (1.5mm thickness) with a pH gradient (Ampholin pH 3.5-10.0). The IEF was carried out with specified running condition according to Hicks *et al.* (1982). The procedure of Vallejos (1983) was followed for staining the peroxidase isozymes.

There was very evident polymorphism in the enzyme peroxidase among the scion bark tissues of the different trees (Fig. 1) despite their genetic homogeneity (see below). While the enzyme polymorphism seen between the rootstock bark tissues (Fig. 1) could be possibly attributed to their genetic divergence (see below), the only plausible explanation for the enzyme polymorphism observed in the scion bark tissues is that the rootstock interfered with the scion because of the large genetic distance existing between them. The exact nature of this interference is unclear, but it is suggested that if the rootstock can affect enzyme polymorphism in the scions (Krishnakumar *et al.*, 1992), probably that could result in metabolic disorders at least in some instances. It has been reported that dissimilarities in the peroxidase isozyme profiles between rootstock and scion can lead to delayed physiological incompatibility in crops such as red oak, Chinese chestnut (Santamour, 1988), which can eventually make the scion vulnerable to diseases (Andrews and Marquez, 1993).

We standardised a suitable protocol for extraction and purification of amplifiable DNA from *Hevea* bark tissues for which extensive trial and error was needed. Unlike young *Hevea* leaves,

bark tissues contain very high concentrations of polysaccharides and phenols which are known to inhibit the Taq polymerase and thus makes it difficult to amplify the DNA in the polymerase chain reaction (Fang *et al.*, 1992).

Bark samples were collected from the rootstock and scion of TPD affected and normal plants from Central Experiment Station, Chethackal and transported to RRII in ice. The samples were washed with sterile water and dried with filter paper. About 0.5g soft bark tissue, wrapped in aluminium foil, was frozen in liquid nitrogen and kept at -60°C for use.

The method as described by Porebski *et al.* (1997) with modifications was used for the extraction of DNA. The bark samples were finely ground using mortar and pestle in the presence of liquid nitrogen. The frozen ground tissue was transferred to 15ml polypropylene centrifuge tubes and mixed with hot (60°C) extraction buffer (100m Tris, 1.4M NaCl, 20mM EDTA, pH 8.0, 2% CTAB, 0.3% B- mercapto ethanol, used fresh) and 50 mg PVP. After mixed with the contents by inversion, the tubes were incubated at 60°C in a water bath for 30 minutes. Chloroform-isoamyl alcohol (24:1v/v, 6ml) was added to the tube when the contents of the tube attained room temperature. After mixed thoroughly, the tubes were centrifuged at 3000 rpm for 20 minutes. The top aqueous phase was transferred into new 15 ml centrifuge tubes using wide-bore pipette tip, and the extraction repeated using chloroform-isoamyl alcohol. Sodium chloride (5M, 1/2 volume of the final aqueous solution recovered) was added to the solution and mixed well, and then two volumes of cold (-20°C) 95% ethanol were added. The tubes were kept at -20°C for 10 minutes after mixed by inversion and then left at 4°C to 6°C overnight. The tubes were spun at 3000 rpm for 6 minutes and the pellet washed with cold (0 to 4°C) 70% (v/v) ethanol. The pellet was dried at room temperature, dissolved in 300 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0) and kept overnight at 4°C to 6°C. The solution was transferred to 1.5ml Eppendrof tube and 3µl RNase A (10 mg/ml) was added and incubated at 37°C for 30 minutes. 300µl neutral phenol was added to each Eppendrof tube, vortexed briefly and spun at 12000 rpm for 10 minutes. The upper layer was collected in fresh 1.5ml tubes and the phenol phase was again extracted with 50 µl TE and added to the sample. The DNA was precipitated by adding 1/10 volume of 2 M sodium acetate and 2 volumes of absolute ethanol and left at -80°C overnight. The tubes were spun at 12000 rpm for 10 minutes and the pellet washed with cold (0 to 4°C) 70% v/v ethanol. The tubes were dried at room temperature and the precipitate was dissolved in 100µl to 200 µl TE by keeping at 4°C for complete resuspension.

DNA concentrations were measured using a Beckman UV- spectrophotometer. The average yield of DNA ranged from 50 to 100 µg/g bark tissue and 10 ng dilutions were prepared. PCR amplifications were carried out using four primers (OPA10, OPD8, OPE1 and OPB15) under the following PCR amplification conditions. Ten picomoles of the primer and 50ng of template DNA were combined with polymerase buffer, 2mM $MgCl_2$, 0.2 mM concentrations of each dNTPS and 0.7 unit of Taq DNA polymerase (Promega, USA) to a final reaction volume of 25 µl. The temperature cycles used were as follows: an initial denaturation at 94°C for 3 minutes, followed by 40 cycle reaction of denaturation at 94°C for 30 seconds, annealing at 36°C for 1 minute, polymerisation at 72°C for 2 minutes and a final extension at 72°C for 7 minutes. PCR amplications were then run in a 1.5% 3 mm thin agarose gel, stained with ethidium bromide and photographs were taken using a UV light source.

Restriction digestion of the DNA samples with *Eco*RI gave total digestion of DNA (Fig. 2A) and repeatability of the PCR amplification of the DNA was established. This indicated that the protocol used gave pure DNA samples which were digestible and amplifiable. The RAPD profile of the bark DNA using the primers OPD8 and OPA10 are given in Figures 2B and 2C, respectively. We calculated the genetic distance between the samples using the modified Jaccard Index (Jackson *et al.*, 1989).

$Gd_{ij} = (B_{ij}/M_{ij})*100$

where Gd_{ij} is the genetic distance between the samples i and j; B_{ij} is the number of polymorphic bands between i and j and M_{ij} is the total number of band positions in i and j.

RESULTS AND DISCUSSION

As expected and unlike the peroxidase enzyme polymorphism, the DNA profile from the scion tissues were identical and the genetic distance between them was zero, because they all came from the same clone, GT1. The DNA profiles from the rootstocks were different, confirming their genetic heterogeneity. The genetic distance between the rootstock tissues ranged from 7% to 39% (Table 1). While changes in the DNA profiles between two trees could be attributed to true genetic differences, the same cannot be said with reference to enzyme polymorphism which is more related to gene expression.

The pooled data of the genetic distance between the rootstocks and scions obtained from the RAPD profiles using all the four different primers were statistically tested using independent t test. The results are given in Figure 3. There is a clear indication that when the DNA was amplified using the PCR technique, the genetic distance was on the higher side in the TPD affected trees than in the normal trees. The estimated genetic distance between the rootstock and scion was higher in the TPD affected trees than in the healthy ones at a probability of 0.07. Apart from the standardisation of the extraction and PCR amplification of DNA from the bark tissues of *Hevea*, the present study is interesting at least in two other different ways. First, we are trying to test an entirely novel hypothesis viz. that the greater the genetic differences between the rootstock and scion tissues, the greater the probability of manifestations of a physiological disorder like TPD. This has direct implications for TPD research as well as much wider ramifications in various aspects of Plant Biology. Secondly, if the initial indications that we obtained in the present study, viz. the association of a greater genetic distance between the rootstock and scion tissues with TPD are in fact real and repeatable for which studies are already under progress in our laboratory using more samples and primers, several new questions need to be addressed. For instance, if TPD is related to the genetic distance between the root-stock and scion, then why do we see TPD in a clonal population where the trees are not bud grafted and therefore no rootstocks are present? In this context it may be noted that the possibility of the existence of different types of TPD which have different causative factors can not be totally ruled out (de Fay and Jacob, 1989). Is that the reason why identification of any single cause for TPD has been elusive for such a long time?

REFERENCES

Andrews, P.K., and C.S.Marquez. 1993. Graft incompatibility. Hort. Rev. 15: 183-232.

Fang, G., S. Hammar, and R.Grumet. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. Biofeedback, 13(1): 52-54.

de Fay, E., and J.L. Jacob. 1989. The bark dryness disease (brown bast) of *Hevea*. In: d'Auzac, J.L. Jacob and H. Chrestin (Eds.). Physiology of Rubber Tree Latex, CRC Press, Boca Raton. pp. 407-409.

Hicks, P.H., D.A.Sleper, D.D. Randall and C.F. Crane. 1982. Peroxidase isozyme differences in tall fescue cultivars and allopolyploid accessions. Euphytica, 31: 175-181.

Jackson, D.A., K.M. Somers and H.H.Harvey. 1989. Similarity coefficient, measures of co-occurrence and association or simply measures of occurrence? American Naturalist, 133: 436-453.

Krishnakumar, R., M.P. Asokan and M.R. Sethuraj. 1992. Polymorphic isozyme expression caused by stock-scion interaction in *Hevea brasiliensis* clone RRII 105. Indian Journal of Natural Rubber Research, 5(1&2): 161-171.

Porebski, S., G.L.Bailey and B.R.Baum. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter, 15(1): 8-15.

Santamour, F.S. 1988. Graft incompatibility in woody plants: an expanded perspective. J.Environ.Hort. 6: 27-32.

Sobhana, P. 1998. Physiology of rooting and stock-scion interaction in *Hevea*. Ph.D. Thesis, University of Kerala, India.

Vallejos, C.E. 1983. Enzyme staining. In: S.D.Tanskley and T.J. Orton (Eds). Isozymes in Plant Genetics and Breeding, Part A, Elsevier, Amsterdam, pp. 469-516.

Yeang, H.Y., R.Wickneswari, E.Sunderasan, S.K.Leang, H.A.Asari, D.M.Napi, A.S.M.Zamri, and M.N.A. Ghami. 1995. Isozymes in *Hevea* crop improvement. 1995 IRRDB Symposium on Physiological and Molecular Aspects of the Breeding of *Hevea brasiliensis*, England.

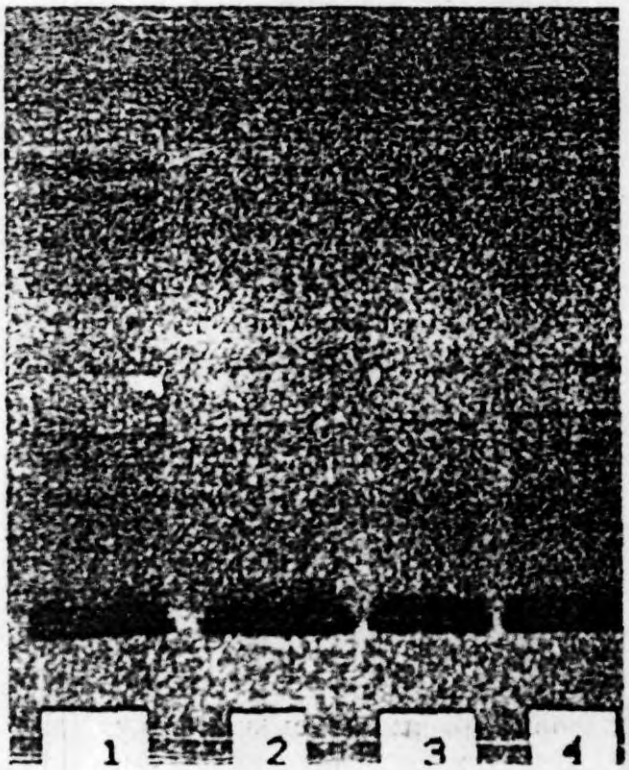

Figure 1. Zymogram showing the peroxidase isozyme of the bark tissue of Clone GT1.

Lanes 1 & 3 represent rootstocks of two trees and lanes 2 & 4 represent their corresponding scions.

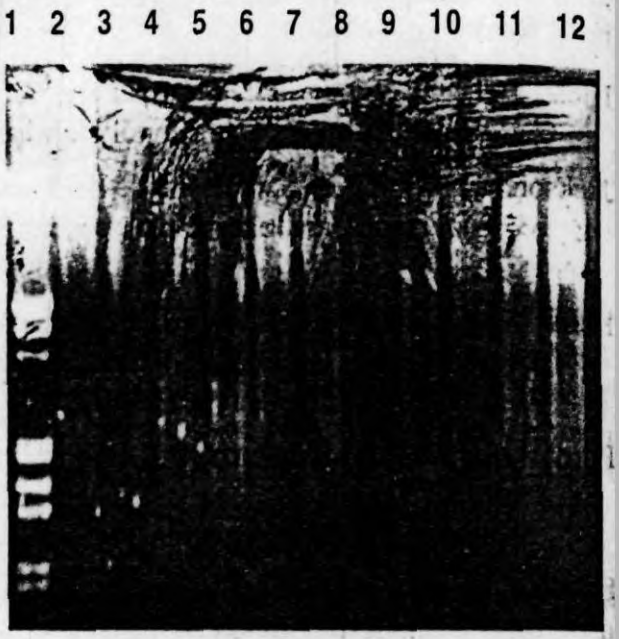

Figure 2A. Restriction digestion of bark DNA samples with Eco RI.

Lane 1: DNA Marker; Lane 2: uncut DNA; Lanes 3-11: DNA samples digested with Eco RI.

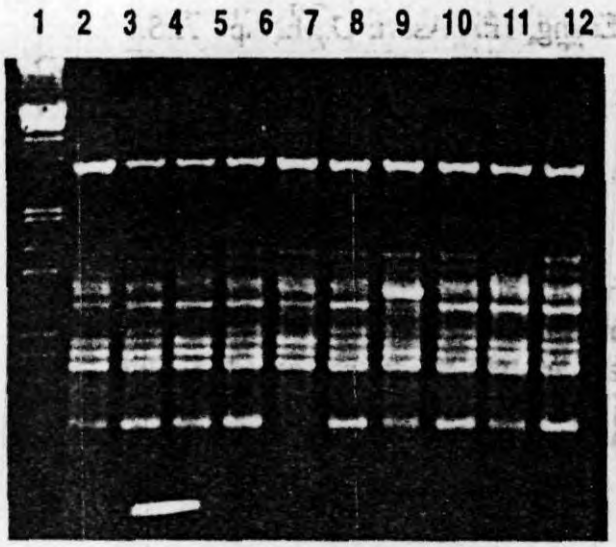

Figure 2B. RAPD profile of the bark DNA (stock and scion) using the primer OPD8.

Lane 1: DNA Marker; Lanes 2 - 5 are normal trees and Lanes 6 - 11 are TPD affected trees. Lanes at even number positions are stock samples and at odd number positions are scion samples; with two adjacent lanes (starting from lane 2) representing the same tree.

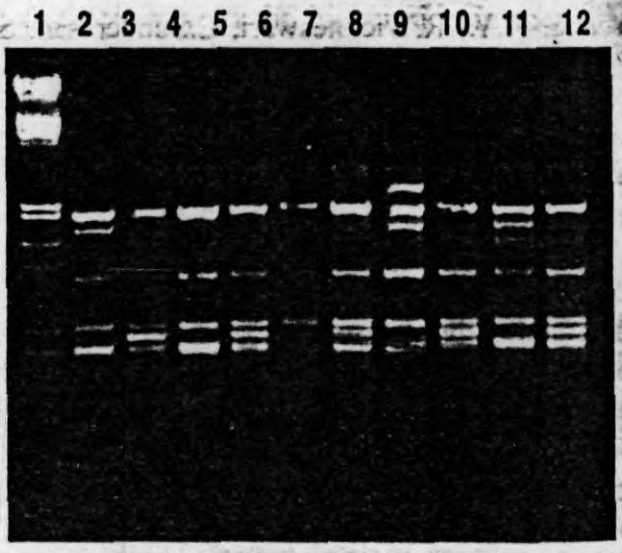

Figure 2C. RAPD profile of the bark DNA using the primer OPA10.

Lane 1- DNA Marker. Lanes 2 - 5 are normal trees and Lanes 6 - 11 are TPD affected trees. Lanes at even number positions are stock samples and at odd number positions are scion samples; with two adjacent lanes (starting from lane 2) representing the same tree.