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Full Length Research Paper

Detection of *Hevea brasilensis* clones yield potential and susceptibility to tapping panel dryness in Côte d'Ivoire using the 32 and 35 KDa lutoidic proteins

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This study was carried out to identify protein markers with yield potential and susceptibility to tapping panel dryness (TPD). To achieve this goal, 11 clones, stimulated and non stimulated, yield and susceptibility to TPD were compared. Their lutoid fraction polypeptides were analysed using one and two-dimensional electrophoresis. Susceptibility to TPD appeared as a clonal trait which is not related to yield potential. TPD can occur either in stimulated or non stimulated clones but, overstimulation increase TPD symptoms. Clones PB 235, PB 260 and IRCA 130 are highly susceptible to TPD, whereas IRCA 41, PB 217, AF 261, AVROS 2037 and GT 1 are less susceptible. Productive and less susceptible clones to TPD are characterized by abundant quantity of 35 KDa polypeptide and absence or very small amount of 32 KDa polypeptide. Contrarily, clone susceptible to TPD are characterized by abundant quantity of 32 KDa polypeptide. In unproductive clones (RO 38, TJR 1), 32 KDa protein was more abundant than 35 KDa. Overstimulation induces a decrease of 35 KDa protein intensity. Thus, 32 and 35 KDa polypeptides could be used for identification of *Hevea* clones yielding potential and susceptibility to TPD.

Key words: *Hevea brasiliensis*, lutoid, protein, rubber, tapping panel dryness, yield.

INTRODUCTION

Hevea brasiliensis is cultivated especially for its latex high rubber content. Tapping induces latex flow out. Latex expelled upon tapping is a cytoplasm from specialized bark cells called laticiferous (Andrews and Dickenson, 1961). As a true cell cytoplasm, latex contains usual organelles of non photosynthetic plant cells (D'Auzac and Jacob, 1989). It also contains lutoids representing the vacuolar and lysosomal system (Coupé, 1977) and more specifically, rubber particles in very large number. Rubber trees yield has been significantly increased due to breeding and the use of ethephon (an ethylene generator) to stimulate latex production (D'Auzac et al., 1989). However, overstimulation could induce an oxidative stress within the latex cells, leading to the syndrome of tapping panel dryness (TPD), resulting in a partial or total cessation of latex flow (Chrestin,

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Abbreviation: TPD, Tapping panel dryness.

Table 1. Some characteristics of the studied clones.

Clone	Origin	Yield	Cultivated surface in estate company (%)
AF 261	Unknown	Medium	1
AVROS 2037	Malaysia	Medium-low	4
GT 1	Malaysia	Medium	25
IRCA 41	Côte d'Ivoire	High	6
IRCA 130	Côte d'Ivoire	High	0.1
IRCA 230	Côte d'Ivoire	High	2
PB 217	Malaysia	High	17
PB 235	Malaysia	High	8
PB 260	Malaysia	High	5
RO 38	Brazil	Low	<0.1
TJIR 1	Malaysia	Low	<0.1

1989). The syndrome seems to be a physiological disorder which can occur naturally, but in most cases, it is induced by the overtapping and/or overstimulation. Rubber plantations are affected by the TPD and this phenomenon is growing in scale. TPD occurs in 12 to 50% of rubber trees in almost every rubber growing region. It leads to the loss of 12 to 14% of the annual rubber production (Ziang and Zhou, 1997; Chen et al., 2003). There is presently no effective prevention or treatment for this serious physiological disease. Response to stimulation and susceptibility to TPD depends on clone (Yan and Fan, 1995). Thus, in Côte d'Ivoire, rubber breeding and selection tend to promote clones with high yielding potential and less susceptibility to TPD. Previous studies have identified in latex cytosol and rubber particle, several putative protein markers that are linked to TPD (Sookmark et al., 2002; Dian 1993; Okoma, 2008). A 35 to 36 KDa protein which seems to be involved in rubber production has been shown in lutoid protein profile of three clones (Koffi, 1995). This study aims at identifing putative yield and TPD makers in latex lutoid fraction. To achieve this objective, yield and TPD susceptibility of 11 clones was determined in correlation with their lutoid protein profile in (1D) and (2D) dimensional (1D) electrophoresis.

MATERIALS AND METHODS

Planting material

This study was conducted using 11 clones of *H. brasiliensis* (Table 1). GT 1 is the first and the most planted clone in Côte d'Ivoire. It is characterized by a medium yield, a good response to stimulation and a low susceptibility to TPD. Clones were planted in the experimental field of the National Center of Agronomic Research (CNRA) at Anguededou, in the south east of Côte d'Ivoire.

Methods

Yield potential assessment

Rubber production was measured on 9 clones grafted in the same trial carried out in 1991. Each clone was represented by ten trees. Since 1998, trees were opened and tapped without hormonal stimulation. In 2004 and 2005, they were stimulated with a mixture of ethrel and oil palm at 2.5% active matter (ethephon). Four stimulations were applied per year.

Susceptibility to TPD determination

Susceptibility to TPD was measured on the same 9 clones. The experimental design was a one tree plot design, with six treatments (T1, T2, T3, T4, T5 and T6) and 33 trees per treatment. In all the treatments, tapping was performed on a S/2 d4 6d/7 (tapping in half spiral downward at a frequency of four times daily, six days in tapping followed by one day rest). T1 (control): Non stimulated trees; T2: ET2.5% Pa1(1) 2/y, stimulated with ethephon with 2.5% active matter, 1 g of stimulant applied on panel on 1 cm band, 2 applications per year; T3: ET2.5% Pa1(1) 4/y, stimulated with ethephon with 2.5% active matter, 1 g of stimulant applied on panel on 1 cm band, 4 applications per year; 4: ET2.5% Pa1(1) 8/y, stimulated with ethephon with 2.5% active matter, 1 g of stimulant applied on panel on 1 cm band, 8 applications per year; T5: ET2.5% Pa1(1) 13/y, stimulated with ethephon with 2.5% active matter, 1 g of stimulant applied on panel on 1 cm band, 13 applications per year; T6: ET2.5 % Pa1(1) 26/y, stimulated with ethephon with 2.5 % active matter, 1 g of stimulant applied on panel on 1 cm band, 26 applications per year.

TPD symptoms were recorded as the percentage of tapping cut length appearing dry upon tapping. Data were recorded every month for three years.

Protein profile analysis

Lutoid protein profile of 11 clones was assessed. Fresh latex collected from ten trees of each clone was mixed. A sample from this mixture was centrifuged at 10 000 rpm for 15 min at 4°C. The bottom fraction was washed three times with a 7.5 pH buffer composed of 50 ml of Tris (50 mM), $MgCl_2$ (5 mM), amonium molybdate (100 µm), mannitol (400 mM) and DTT (1 mM), and then subjected alternatively to freezing and thawing three times, followed by centrifugation to obtain lutoid protein. Total protein from each clone latex fraction was quantified using protein micro assays based on Sedmak and Grossberg (1977) method. Proteins were separated by one(1D) and two(2D) dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). A 12.5% acrylamide gel was performed using a vertical gel system (Bio-Rad Protean II Slab Cell). 1D-SDS-PAGE was prepared according to

Table 2. Yield assessment during two years, before and after stimulation, in 9 clones out of 11 clones studied.

Clone	Yield ($ga^{-1}s^{-1}cm^{-1}$)			
	Before stimulation (% GT1)		After stimulation (% GT1)	
PB 235	3.1[a]	(246%)	4.1[a]	(290%)
IRCA 130	3.0[ab]	(232%)	2.6[bc]	(183%)
PB 217	2.3[ab]	(184%)	3.8[ab]	(268%)
IRCA 230	2.0[abc]	(160%)	2.5[bc]	(178%)
PB 260	1.8[bc]	(139%)	3.5[ab]	(248%)
GT 1	1.3[c]	(100%)	1.4[cd]	(100%)
AVROS 2037	0.8[cd]	(63%)	2.3[bc]	(167%)
TJIR 1	0.7[cd]	(53%)	0.9[cd]	(67%)
RO 38	0.3[d]	(25%)	0.6[d]	(40%)

Means followed by the same letter in each column are not significantly different (test of Newman-Keuls at 5%).

Table 3. Comparison of the susceptibility to TPD over the two years of study in 9 clones out of the 11 clones tested.

Clone	TPD (%)	Susceptibility to TPD
PB 235	6.3[a]	
PB 260	5.4[ab]	High
IRCA 130	4.0[abc]	
IRCA 230	2.3[bc]	Intermediate
AF 261	1.3[c]	
GT 1	1.3[c]	
AVROS 2037	1.0[c]	Low
PB 217	0.5[c]	
IRCA 41	0.2[c]	

Means followed by thevsame letter in each column are not significantly different (test of Newman-Keuls at 5%).

Laemmli (1970). The proteins were separated by 2D-SDS-PAGE, following a method adapted from O'Farrell (1975). Equal amounts of protein of each clone were loaded on the gel, run at 12 mA for 8 h and silver stained according to Helmut et al. (1987).

Identification of polypeptides related to TPD was made by visual analysis of qualitative and quantitative variations observed on the clones protein profiles.

Statistical analysis

Yield and TPD data were subjected to analysis of variance (ANOVA) using statistics software XLSTAT. The level of significance of the differences between averages was estimated by the Newman-Keuls test at a limit of 5%.

RESULTS

Classification of clones according to yield potential

Stimulated and non stimulated yield of clones PB 235, IRCA 130, PB 217, IRCA 230 and PB 260 were higher than that of GT 1. Hormonal stimulation induced an increase in the yield of all the clones except IRCA 130. The yield of this clone decreased from 232 to 183% of the one of GT 1 after two years of stimulation. TIJR 1 and RO 38 were the least productive of the studied clones. In spite of stimulation, their yield remained lower than that of GT 1. High yielding clones where PB 235, IRCA 130, PB 217, IRCA 230 and PB 260, whereas low yielding were RO 38 and TJIR 1 (Table 2).

Evolution of clones for susceptibility to TPD

Over the two years of study, the occurrence of TPD varies considerably from one clone to another. The highest susceptibility to TPD was recorded in clones PB 235, PB 260, and IRCA 130. Contrarily, the weakest susceptibility to TPD was observed in clones IRCA 41, PB 217, AVROS 2037, GT 1 and AF 261. IRCA 230 was intermediate between low and high susceptible clones (Table 3).

Both non stimulated and stimulated clones presented the syndrome of TPD. In addition, in almost all clones, the tapping panel dryness observed on tapping cut length of trees stimulated 2 or 4 times per year were lower or equivalent to those from non stimulated ones. This tapping panel dryness increased with the frequency of stimulation. The tapping panel dryness in IRCA 130 increased significantly after two stimulations per year, whereas in clones PB 260 and PB 235, this part increased beyond 4 stimulations per year. In clones IRCA

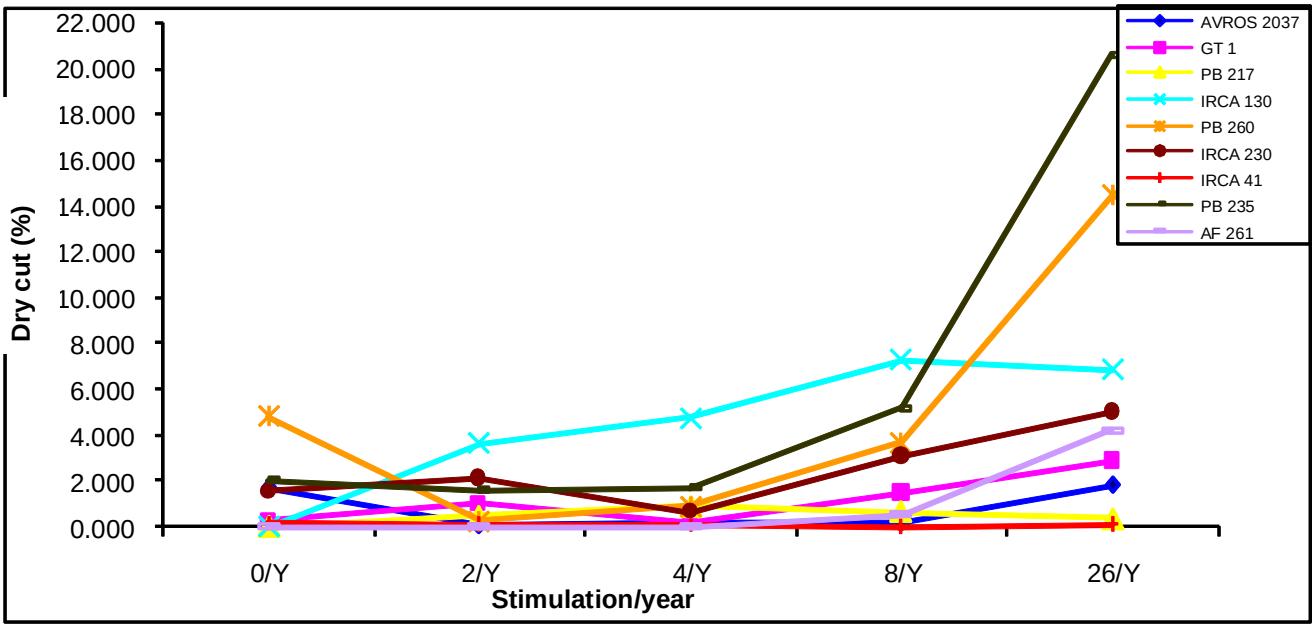

Figure 1. Evolution of the dry cut over two years according to the number of annual stimulation in 9 clones out of 11 clones tested.

41 and PB 217, the tapping panel dryness remained weak after 26 stimulations (Figure 1).

Determination of clone susceptibility to TPD by lutoid proteins profile analysis

The 32 KDa protein was abundant in lutoid profile of clones highly susceptible to TPD (PB 235, PB 260 and IRCA 130). In contrast, this protein was absent or very weak in lutoid profile of clones weakly susceptible to TPD (IRCA 41, PB 217, AF 261, GT 1 and AVROS 2037). Comparing high yielding potential clones (PB 235, PB 260 and IRCA 130) with unproductive ones as RO 38 and TJIR1, the 32 KDa protein was more abundant than that of 35 KDa (Figure 2).

Two-dimensional-SDS-PAGE showed 2 polypeptides of 35 KDa called 35 KD1 and 35 KD2. Over stimulation induced a decrease of 35 KD1 and 35 KD2 protein intensity in susceptible and non susceptible clones. However, this reduction was more significant in highly susceptible clone. The 35 KD1 seems to disappear in the highly susceptible clone PB 235 to TPD when it was overstimulated, whereas it remained perceptible in the low susceptible clone PB 217 during overstimulation (Figures 3 and 4).

DISCUSSION

The studied clones presented different levels of susceptibility to TPD. The clones PB 235, IRCA 130 and PB 260 were highly susceptible to TPD, whereas IRCA 41, PB 217, AVROS 2037, GT 1, AF 261 and IRCA 230 were less susceptible. Yan and Fan (1995) have also reported that *Hevea* clones showed different levels of TPD severity. The TPD appeared in non stimulated clones as well as in stimulated ones. This implies that hormonal stimulation could not be the only factor related to the occurrence of TPD. Some studies suggested a biotic origin of TPD (Zheng et al., 1988; Ramachandran et al., 2000) but it is commonly admitted that in most cases, TPD is induced by the over-tapping and/or over-stimulation (Jacob et al., 1994; Faridah et al., 1996). The dry cut length increased with the frequency of stimulation. In clone IRCA 130, the dry cut increased considerably starting from two stimulations per year. With clones PB 235 and PB 260, this increase started from four stimulations per year, whereas with clones IRCA 41 and PB 217, this dryness remained low after 26 stimulations. This result shows the existence of a tolerance level to stimulation for each clone. In spite of their high yield, clones IRCA 41, PB 217 and IRCA 230 were less susceptible to TPD than the other high yielding clones (PB 235, PB 260 and IRCA 130). This suggested that sensitivity to TPD is a clonal-related trait which is not dependent on yield level. The study of lutoid proteins profile in relation to clonal susceptibility to TPD using one and two-dimensional electrophoresis revealed a distinguishable polymorphism related to rubber production and sensitivity to TPD in 35 and 32 KDa proteins. The 32 KDa protein was more abundant than 35 KDa protein in low yielding clones. Contrarily, 35 KDa protein was more abundant than 32 KDa protein in high yielding clones. The 35 KDa protein, especially 35 KDa 1, tends to disappear when clones are over stimulated. In these

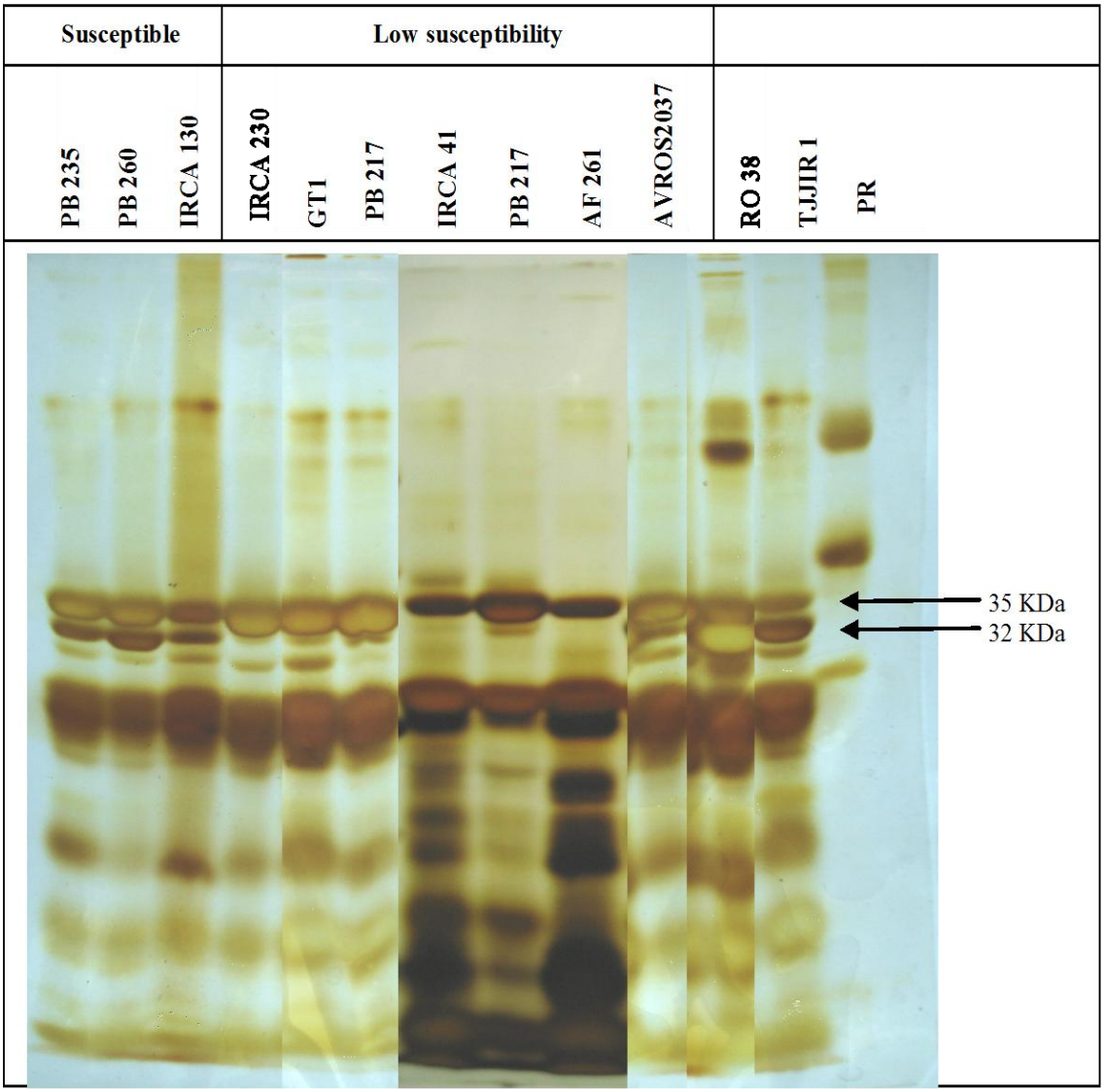

Figure 2. Representative 1-D SDS PAGE gels of *H. brasiliensis* latex lutoi protein when comparing tolerant and susceptible clones with TPD. 1-D SDS PAGE: one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis; PR: protein reference.

conditions, these clones express TPD symptoms and become less productive. Clone highly susceptible to TPD were characterized by an abundance of 32 KDa protein. The abundance of 32 KDa seems correlated with a poor resistance to the stress caused by hormonal stimulation. The abundance of 35 KDa, in spite of overstimulation, and the absence or a weak amount of 32 KDa proteins seems to be an indicator of high yield potential which results in a high stimulation acceptance. It was shown that lutoid protein with molecular masses of about 35 and 32 KDa are respectively, glycoprotein and non glycosylate isozymes of 1,3-β-glucanase (Vögeli-Lange et al., 1994; Churngchow et al., 1995). It has been suggested that 1,3-β-glucanase is important for diverse physiological processes, such as, defence against pathogens and insect, stress response, and plants mobilisation of store reserves (Vögeli-Lange et al., 1994; Thanseem et al., 2005).

The lutoidic proteins of 35 and 32 kD, appeared as markers of yield potential and TPD susceptibility. The 35 kD protein seems to influence positively yield, whereas the 32 kD protein affects the latter negatively.

The abundance of the 32 kD protein seems to express a sensitivity to the stress caused by hormonal stimulation. If the correlation between TPD, yield and protein profiles observed on old trees are perceptible in nursery, 35 and 32 KDa protein could become criteria for early selection of high yielding clones less susceptible to TPD.

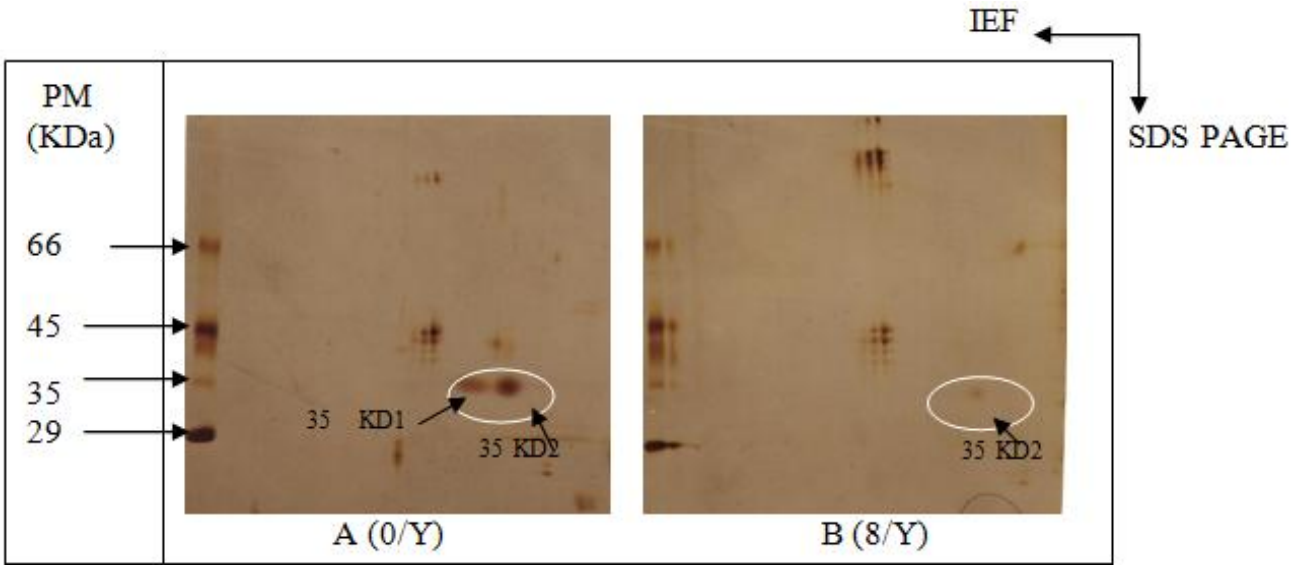

Figure 3. Representative 2-D SDS-PAGE of lutoid protein of clone highly susceptible to TPD (PB 235) non stimulated (A) and stimulated 8 times per year (B). 2-D SDS PAGE: Two dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis.

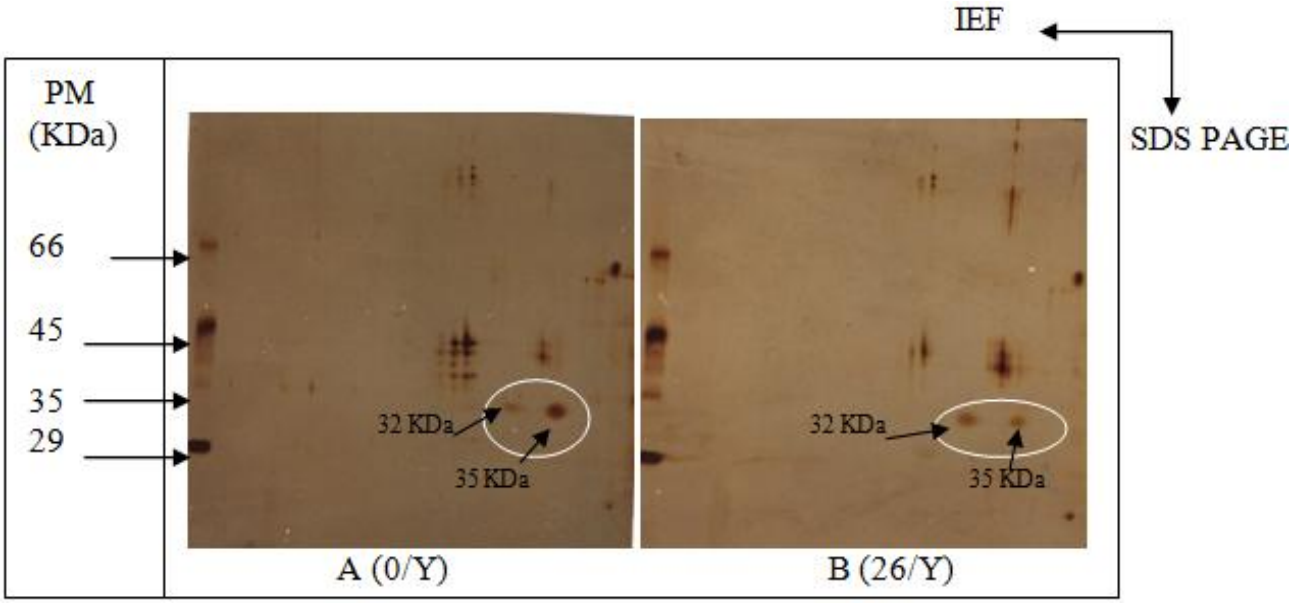

Figure 4. Representative 2-D SDS-PAGE of lutoid protein of clone less susceptible to TPD (PB 217) non stimulated (A) and stimulated 26 times per year (B). 2-D SDS PAGE: Two dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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