Research Article

doi:10.19071/jpc.2015.v43.i3.2854
Journal of Plantation Crops, 2015, 43(3): 204-211

Physiological comparison of root trainer and polybag plants of *Hevea brasiliensis*

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(Manuscript Received:03-07-2015, Revised:11-09-2015, Accepted:12-10-2015)

Abstract

The physiology of root trainer plants in comparison to polybag plants of two popular *Hevea* clones, RRII 105 and RRII 430 was studied. Dry matter partitioning and physiological parameters like leaf water potential, relative water content, chlorophyll content, PS II activity and photosynthetic efficiency were studied in root trainer and polybag plants and compared. Root trainer plants had only 55 per cent less biomass than polybag plants at single whorled stage. No variation was noticed in water relations among the polybag and root trainer plants. Total chlorophyll content and chlorophyll *a/b* ratio were higher in polybag plants than root trainer plants of both clones. Polybag plants showed higher light saturation point when gas exchange was measured at different photosynthetic photon flux densities. Though there was no variation in effective quantum yield (Φ PSII), the root trainer plants of RRII 105 showed more excess electrons (J*) at higher light intensities. Photosystem II activity and photosynthetic carbon assimilation rates were less in root trainer plants under open and shaded conditions. The results indicated that the root confinement in root trainer plants has significant effect on the physiology of plants. The reduction in plant biomass and other physiological traits in root trainer plants can be attributed to the limited space in the container, limiting further root growth, which otherwise is beneficial for giving a priming effect as long as the plant remains in the container. Further studies are needed to quantify this 'stress factor' in root trainer plants and its implications in growth and establishment of plants in the field.

Keywords: Photosynthesis, polybag, PS II activity, root trainer, stress

Introduction

Planting materials of *Hevea* are traditionally raised in polybags, and in such plants, as soon as the roots reach the lower end of the polybag, strangling and distortion occur due to root coiling (Ginwal *et al.*, 2001; Soman and Saraswathy Amma, 1999). Another major disadvantage of polybag plants is during long distance transport; there are chances of the soil core and the root system getting damaged. Raising plants in root trainer containers is a cost-effective, eco-friendly and labour-saving propagation technique, gaining popularity in many rubber growing countries. A root trainer container is made up of polypropylene, with a holding capacity of 600-800 cm^3. The growth medium used in root trainer contains cured coir pith, rock phosphate, neem cake, bone meal and plant protection chemicals (Soman *et al.*, 2013). After filling the potting medium, the root trainers are stacked in trenches and germinated seeds are planted. The plants, on attaining sufficient growth, are budgrafted and successful budgrafts are cut back when stock seedlings attain four months growth. When the scion attains sufficient growth, the plants are lifted from the trench, outgrowing roots are pruned and plants are kept on stands under shade for hardening in such a way that the root trainer is suspended in air. During the hardening process, the tap root resumes growth and undergoes natural air pruning near the hole at the bottom, and thus prevents its coiling inside the container (Soman *et al.*, 2011). Root trainer grown plants constantly experience mild 'stress' due to the self-pruning of tap root, the tip of which is in contact with air and this leads to the emergence of numerous lateral roots into the well-aerated potting medium. The hardened root trainer plant will have a root

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**Corresponding Author: sumesh@rubberboard.org.in* system consisting of a central tap root and well oriented lateral roots without any deformity (Mydin *et al*., 2010). Plants raised in root trainers showed better sturdiness (height:diameter ratio) and uniform distribution of roots than polybag plants (Soman *et al.,* 2002). The lateral roots were also found to be significantly higher in root trainer plants than polybag plants (Soman and Saraswathy Amma, 2005). Another advantage is that it is easy to handle while transporting and bulk materials can be transported at a time without causing any damage to the plants.

Root trainer plants will be the preferred planting material in the future owing to its several advantages in the nursery stage. Detailed study on the physiology of root trainer rubber plants have not been conducted so far. The present study, therefore, compares the root trainer and polybag *Hevea* plants and evaluates the photosynthetic performance of these planting materials at two different light levels using two popular rubber clones.

Materials and methods

Planting material

Two types of planting materials, *viz.,* polybag and root trainer plants of two popular clones, RRII 105 and RRII 430 were used for this study. For comparison, plants with same number of whorls were selected. The polybag plants were raised at Rubber Research Institute of India (RRII), Kottayam in 25x55 cm size polythene bags and root trainer plants procured from Rubber Board Central Nursery, Karikkattoor, Pathanamthitta, Kerala were raised in root trainers of capacity 600 cm^3 and 26 cm length. These planting materials were maintained at RRII since August 2013. One set of polybag and root trainer plants (10 plants each) were grown under normal conditions in the open sunlight. Another set of 10 plants were maintained under partial shade (30-35% shade) throughout the study period to analyse the photosynthetic response under low light in these plants. The plants were irrigated regularly and other management practices were followed for each planting material as per recommended practices.

Dry matter partitioning, water relations and chlorophyll content

The dry matter of root trainer and polybag plants were calculated gravimetrically, after drying the plant parts in a hot air oven at 70 ^{0}C until constant weight. Water potential (Ψ_L) was determined at 9.00 am on excised leaf discs by using Psypro Water Potential System (Wescor, Logan, USA). Relative water content (RWC) was determined in the fully expanded leaf from the top whorl of the main shoot according to Barrs and Weatherly (1962). The leaf chlorophyll (chl) was extracted in dimethyl sulphoxide:acetone (1:1) solution and total chl content was estimated by the method of Arnon (1949) modified by Hiscox and Israelstam (1979).

Chlorophyll fluorescence and gas exchange

Light response (P_N/I) curves from fully mature leaves of plants adapted to laboratory conditions for two days were measured with a portable photosynthesis system (Li-6400XT, Li-Cor, USA) coupled with a leaf chamber fluorometer and a CO$_2$ mixer. The measurements were taken at 60-70 per cent relative humidity and 400 µmol (CO$_2$) mol (air)$^{-1}$ CO$_2$ concentration inside the chamber. P_N/I curve was derived using the Li-6400XT with a sequence of light settings (1900, 1500, 1000, 800, 600, 400, 200, 100, 50, 20 and 0 µmol (photon) m^{-2} s^{-1}), minimum wait time of 120 s, maximum wait time of 200s, and matching the infra-red gas analyzers for 50 µmol (CO$_2$) mol (air)$^{-1}$ difference in the CO$_2$ concentration between the sample and the reference chambers, which allowed them to be matched before every change in *I*. P_N/I curves, were fit using the Excel Routines developed by Lobo *et al*. (2013) based on the models that were used to fit P_N/I curves. Rate of excess electrons (J*) was calculated by subtracting the rate of electron flow to CO$_2$ assimilation from the rate of non-cyclic electron flow across PS II.

Photosynthetic rate (P$_N$), stomatal conductance (g$_s$) and transpiration rate (E) were measured using LI-6400XT portable photosynthesis system, at a reference CO$_2$ of 400 µmol (CO$_2$) mol (air)$^{-1}$ and light intensity 500 µmol m^{-2} s^{-1}. Measurements were done in intact, mature, fully expanded leaves (Alam *et al*., 2005). The chlorophyll *a* fluorescence parameters were measured using portable chlorophyll fluorometer, PAM 2100 (Walz, Germany). The leaves were dark/light adapted for 20 minutes by fixing special light-exclusion clips to the leaf surface for Fv/Fm or Φ PSII measurements (Schreiber, 2004).

The mean values (±SE) were calculated for 10 plants of each clone and independent t-test was done to find out the significant difference between the mean values.

Results and discussion

A single whorled root trainer plant had only 55 per cent less biomass (on dry weight basis) than polybag plants, irrespective of the clones (Fig. 1). The shoot growth of root trainer plants was less compared to the polybag plants. This was evident from the smaller leaf size and lesser number of whorls in root trainer plants than polybag plants (Table 1). The root:shoot ratio of plants grown in root trainer and polybags indicated very little variation, irrespective of the type of containers. When the weight of main stump was not considered, the root:shoot ratio of root trainer plants was higher (1 to 1.2) compared to polybag plants (0.8) indicating better lateral root growth compared to shoot growth in root trainer plants. The results indicate that in spite of the better root system, due to the limited space, the plant could not produce equally good shoot system. In a similar study conducted in *Leucaena*, growth of stem and root, collar diameter, number of nodules and the biomass

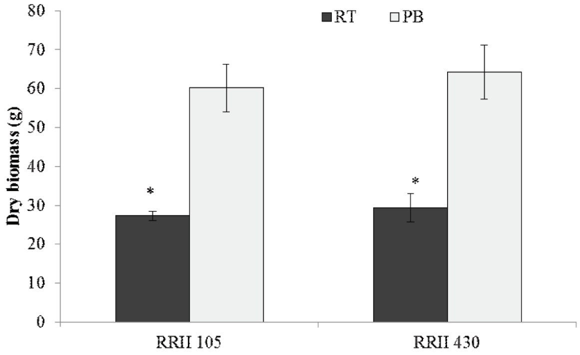

Fig. 1. Total dry matter (shoot+root) present in a single whorled root trainer (RT) and polybag (PB) plants of two clones, RRII 105 and RRII 430 (n=6). * significance at P≤0.05

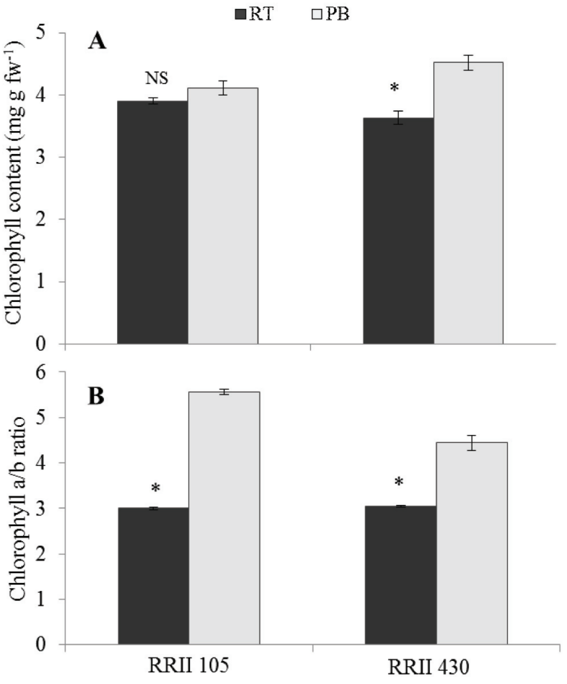

Fig. 2. (A) Total chlorophyll content and (B) Chlorophyll *a*/*b* ratio of two whorled PB and RT plants of RRII 105 and RRII 430 grown under open sunlight condition. * significance at P≤0.05; NS-not significant

Table 1. Growth analysis and dry matter partitioning in single whorled root rainer (RT) and polybag (PB) plants of *Hevea*

Treatment	Plant height from bud union (cm)	No. of leaves	Total leaf area (cm^2)	Shoot biomass (g)	Root biomass (g)	Root: shoot ratio	Root: shoot ratio excluding main stump
RRII 105							
RT	24.2	8.2	454.1	11.9	14.8	1.2	1.0
PB	32.8	9.8	745.3	25.4	34.8	1.4	0.8
CD (5%)	3.3	1.1	154.7	5.6	10.3		
RRII 430							
RT	21.2	7.3	409.2	11.9	17.2	1.4	1.2
PB	30.5	9.8	852.7	28.3	35.9	1.3	0.8
CD (5%)	3.0	1.7	234.1	8.3	9.5		

Table 2. Light response curve derived variables of two clones grown in root trainer (RT) and polybag (PB) containers

Variables	RRII 105 (PB)	RRII 105 (RT)	RRII 430 (PB)	RRII 430 (RT)
I_{comp} (µmol m^{-2} s^{-1})	14.6	13.2	11.7	15.2
R_D (µmol m^{-2} s^{-1})	1.1	1.0	0.9	1.1
P_{gmax} (µmol m^{-2} s^{-1})	11.8	10.1	13.7	11.6
I_{max} (µmol m^{-2} s^{-1})	519	446	607	530
$P_{N(I max)}$ (µmol m^{-2} s^{-1})	9.5	7.7	11.7	9.3

I_{comp} : Light compensation point
R_D : Dark respiration
P_{gmax} : maximum gross photosynthetic rate
I_{max} : light saturation point beyond which there is no significant change in P_N
$P_{N(I max)}$: maximum photosynthetic rate obtained when $I = I_{max}$

parameters were poor which was attributed to the limited space and substratum available to the root system in root trainers in comparison to plants raised in polybags and nursery bed (Ferdousee *et al.*, 2010).

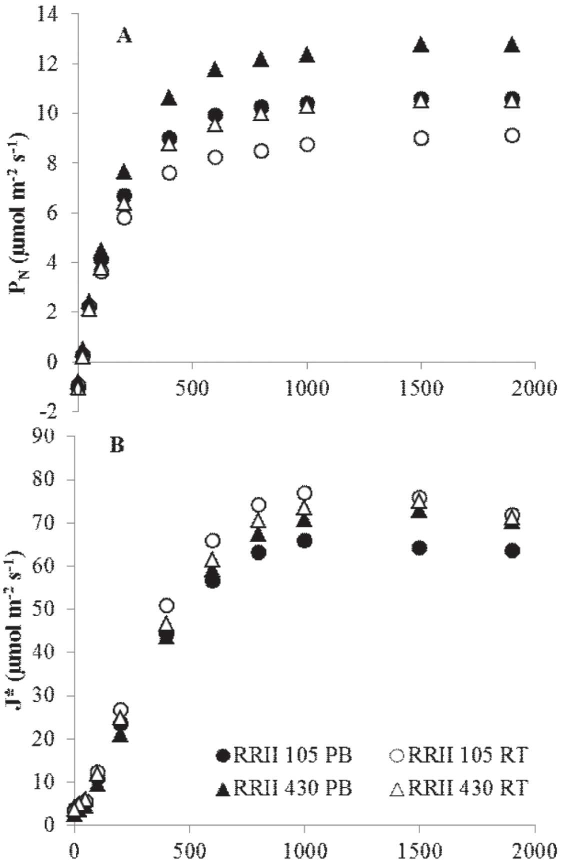

Fig. 3. Rates of (A) net photosynthetic rate and (B) excess electrons versus PPFD in leaves of root trainer and polybag plants of two *Hevea* clones. RT-root trainer; PB-polybag (values are average of 4 independent readings)

Water relations

No significant difference was observed in Ψ_L between root trainer and polybag plants of both the clones (data not shown). RWC values were also insignificant in these plants. Since these plants were irrigated daily, the plant water status in the root trainer and polybag plants did not show any variation indicating that, irrespective of the container type, the water status in all the plants remained same.

Chlorophyll content

The photosynthetic pigments chl *a*, chl *b* and total chl content in the leaf were estimated in polybag and root trainer plants. Clone RRII 430 raised in root trainers showed significantly less total chl content compared to polybag plants in open sunlight (Fig. 2A) while the reduction in total chl was not significant in root trainer grown RRII 105 compared to its polybag plants. Irrespective of clones, chl *a/b* ratio was higher in polybag plants than in root trainer plants (Fig. 2B). RRII 105 had higher chl *a/b* ratio (5.6) than RRII 430 (4.4), however, root trainer plants of both clones had a chl *a/b* ratio of 3.0. The low chl *a/b* ratio observed in root trainer plants can be attributed to their acclimation to the low light condition in which they were raised initially during nursery establishment. A low chl *a/b* ratio in plants indicates increased partitioning of chlorophyll into light-harvesting complexes (Evans and Poorter, 2001), thereby increasing the efficiency of light harvesting under

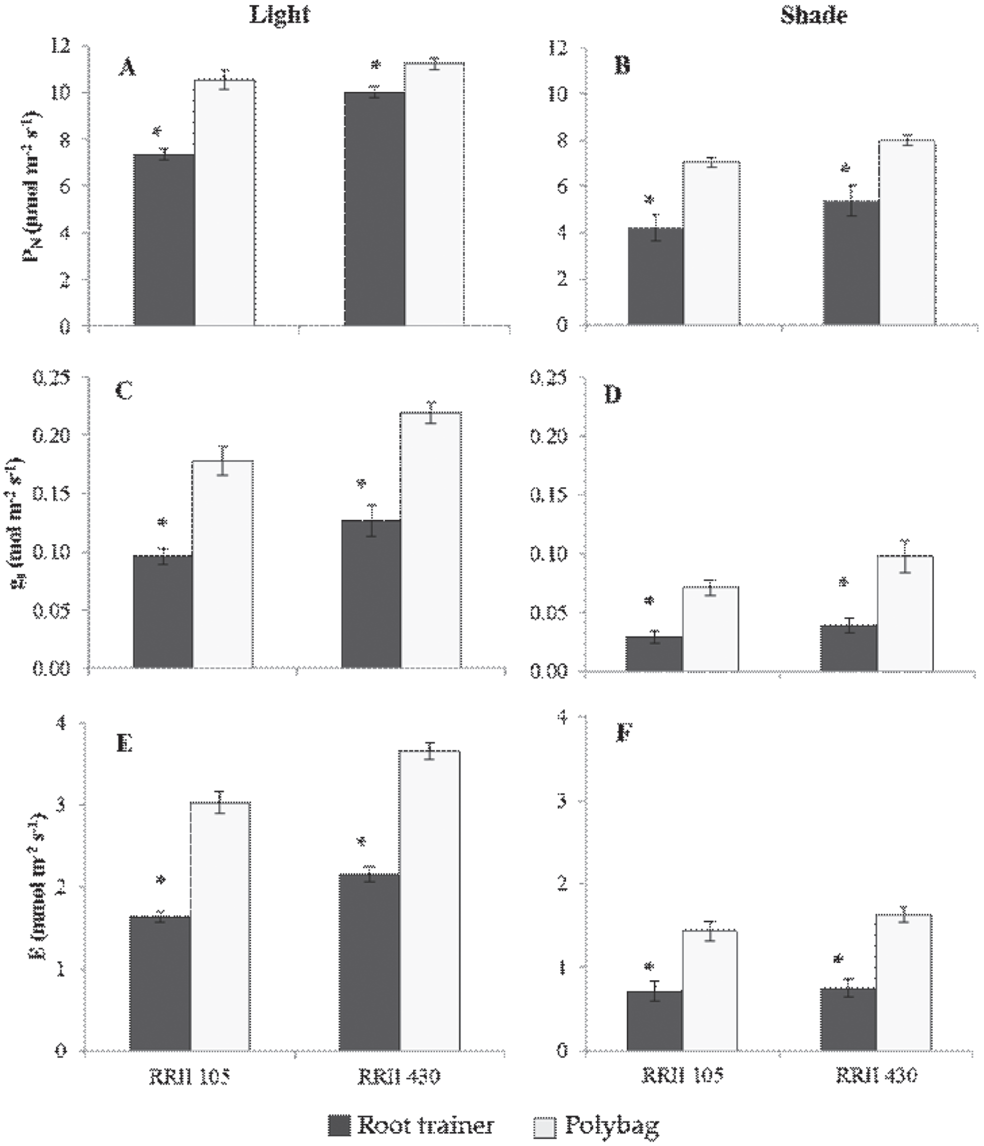

Fig. 4. Photosynthetic rate, stomatal conductance and transpiration rates of clones RRII 105 and RRII 430 grown in polybags and root trainers (A, C & E under light and B, D & F under shade). * indicates significance at $P \leq 0.5$

low-light environments as chl *b* is associated with antenna pigment complexes. A uniformly low chl *a/b* in shade leaves independent of leaf N content was reported by Kitajima and Hogan (2003). It has been shown that shade tolerant species produce a higher proportion of chl *b* relative to chl *a*, which leads to a lower chl *a*/*b* ratio, to enhance the efficiency of blue light absorption in low light environments.

Photosynthetic performance of root trainer and polybag plants

Light response curves clearly indicated that polybag plants are better adapted to high light since the light saturation point was higher for polybag plants than the root trainer plants of both clones (Table 2; Fig. 3A). The maximum gross photosynthetic rate (P_{gmax}) was higher for root trainer as well as polybag plants of RRII 430 than

RRII 105. Excess electrons generated were always more in the case of root trainer plants after a light level of 200 µmol m^{-2} s^{-1} (Fig. 3B) especially in RRII 105, indicating that root trainer plants of this clone is more 'stressed' than RRII 430. The adaptability of modern clone RRII 430 in terms of early establishment and stress survival in the field and nursery conditions under different agro-climate is well documented (Annamalainathan *et al*., 2010; Sumesh *et al*., 2011).

The photosynthetic rate (P_N) was always lower in root trainer than polybag grown plants in both clones (Fig. 4). Polybag plants of RRII 105 and RRII 430 recorded significantly higher photosynthetic rates than their root trainer plants. Among root trainer plants, RRII 430 recorded better photosynthetic rate compared to RRII 105. Under shaded conditions also, polybag plants had significantly better CO_2 assimilation rates compared to root trainer plants. Reduced photosynthetic rate of root trainer grown plants can be attributed to end-product inhibition of photosynthesis caused by root restriction as reported by Schaffer *et al*. (1996) in banana. Starch and other non-structural carbohydrates such as glucose and hexoses might have accumulated in the leaves as a result of sink (root) restriction which has to be explored further. The support for fast growth of shoot meristem and utilization of photosynthates might be restricted by limited soil and rooting medium.

The stomatal conductance in root trainer plant was significantly lesser than polybag plant in both clones (Fig. 4). RRII 430 had better stomatal conductance than RRII 105. Polybag plants of both clones had significantly higher stomatal conductance under shade than the root trainer plants. Transpiration rate (E) showed a similar trend as stomatal conductance in both the clones. Ouma (2007) in a similar study in avocado showed that larger container sizes showed higher CO_2 assimilation rates and stomatal conductance and *vice versa.*

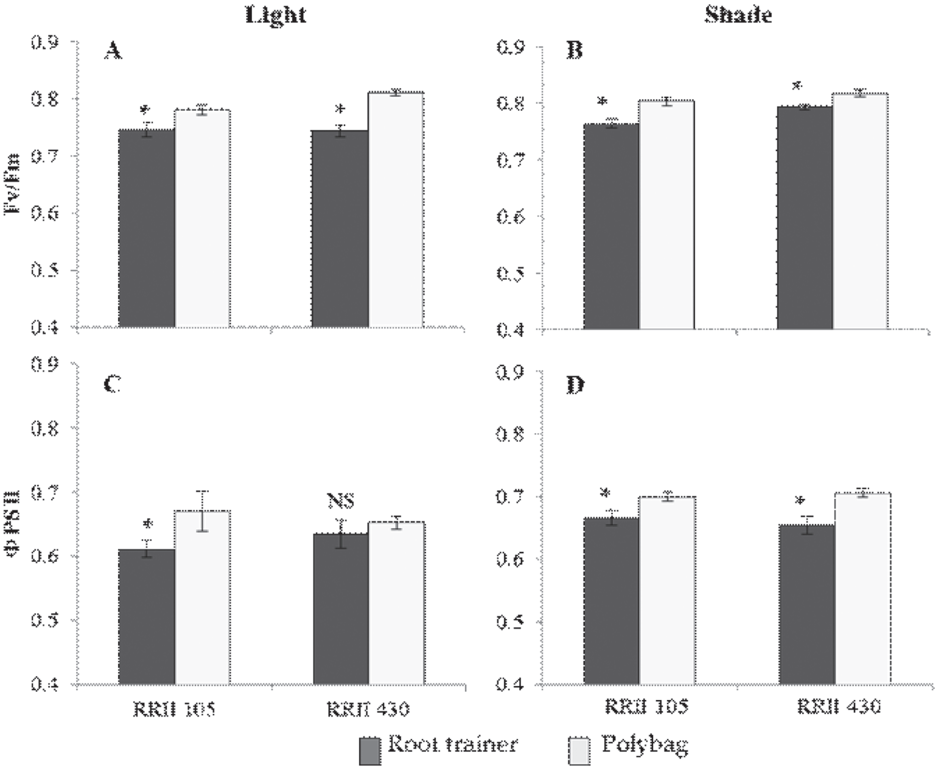

Fig. 5. Maximum (Fv/Fm) and effective (Φ PSII) quantum yields of clones RRII 105 and RRII 430 grown in polybags and root trainers (A&C under light and B&D under shade). * indicates significance at P<0.05; NS - non significant

Chlorophyll fluorescence studies indicated a decreased PS II activity in root trainer plants than polybag plants (Fig. 5). Maximum potential quantum yield of PS II (Fv/Fm) was significantly higher in polybag plants of both clones under open light and shaded conditions. The effective quantum yield (Φ PS II) showed a significant reduction in root trainer plants compared to polybag plants. Φ PS II which gives an indication of PS II activity under a given light level was higher under shaded conditions than at open light in both the clones. Significant differences in Φ PS II was observed between root trainer and polybag plants of both clones under shaded condition, but in the open light, only RRII 105 showed significant difference. These results indicated that the 'stress' experienced by the root trainer plants is also reflected in the light harvesting mechanism. In spite of the low chl *a/b* ratio in root trainer plants, there was no significant partitioning of photosynthetic electrons for photochemistry.

The results indicated that the 'stress' generated by root restriction and air-pruning of tap roots in root trainer plants has significant effect on adaptation features of these plants as long as they remain in the container, which may help the plants to survive better in the field under harsh environments as a result of priming. Further studies under stress environment in the field will help to assess the stress tolerance potential of these plants as a result of root priming or root confinement.

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