

# Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment

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**Abstract** We tested the hypothesis that fertilized containerized *Pinus canariensis* seedlings increases survival when planted in semiarid sites through the improvement of their physiological status during the establishment phase by an increment in root growth. Seedlings were cultured under two different regimes: traditional (in non-fertilized natural soil) and alternative (in fertilized peat). Morphological attributes and nitrogen content were measured before planting. Measurements of survival and growth in the plantation were made periodically for 2 years and physiological plant responses (leaf water potential, gas exchange and chlorophyll fluorescence) during the third summer after planting were tested and finally a set of plants were excavated to

measure the same parameters as before planting. Seedlings cultivated using fertilized peat achieved the highest values for all of evaluated parameters. During the third dry season, big seedlings exhibited better physiological status. Therefore, enhanced root growth can result in better water uptake during the dry period thereby increasing survival and growth in the next few years after planting. A feed-back physiological model is proposed to explain *P. canariensis* establishment in a semiarid environment.

**Keywords** Canary Island pine · Fertilization · Feed-back model · Field performance · Seedling quality · Physiological status

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## Introduction

The fight against desertification in Mediterranean environments involves many strategies for tending to the ecological restoration of degraded lands. Afforestation of former woodlands is a key task to revert the degradation of Mediterranean forests by wildfires, cutting, grazing or soil erosion. However, the achievement of successful plantations has many technical problems imposed by the extreme aridity during summer. Determining the optimum characteristics features of the planting stock for such conditions is crucial.

*Pinus canariensis* is an endemic species in the Canary Islands. Natural pine forests in the Canary Islands grow under different climatic conditions with respect to elevation and geographical position. For example, in Tenerife the distribution limit is between 800 and 2,200 m a.s.l. on north facing slopes and from 500 up to more than 2,500 m a.s.l. on south exposed slopes. (Fernández-Palacios and de-Nicolás 1995). A common feature of these pine forests in

Tenerife is a Mediterranean climate with strong seasonal changes in water availability and evaporative demand (Peters et al. 2003), especially on south exposed slopes where the annual sum of precipitation can be less than 300 mm. Although these pine forests are essential for the local forestry and contribute significantly to the water balance of the island by fog interception they are endangered by forest fires as for example in 2007 where large areas (aprox. 17.000 Ha) of the pine forest have burned down (MMA 2008). Thus, the achievement of a successful re-forestation is crucial for the reversion of these dreadful conditions, which are exacerbated by extreme aridity during the summer months.

In Tenerife, *P. canariensis* nursery crops had been traditionally cultured with natural soil and no additional fertilization, and thus seedlings are in general small in size with a low root biomass (Luis et al. 2004, 2006) and afforestation managers commonly use such seedlings when planting in dryer sites, claiming a superior water balance is better. Conversely, several studies report on better survival rates of fertilized *Pinus* seedlings (Mexal and Landis 1990; Cortina et al. 1997; South 2000; Villar-Salvador et al. 2000; Puértolas et al. 2003) due to a higher root growth capacity (Villar-Salvador et al. 2000). Seedlings are planted during the wet autumn/winter period that can become dry in early spring in the driest sites. Therefore, increased in root growth can result in better water uptake during the dry period thereby increasing survival and growth increment in the next few years after planting. Unfortunately, in spite of the numerous works relating initial seedlings features and survival, there are no studies on the physiological behaviour (WUE or photosynthetic performance) of fertilized seedlings and this assessment has never been tested for *P. canariensis* seedlings.

We hypothesized that *P. canariensis* fertilized seedlings (Alternative culture method) will be bigger and root growth will be improved, and also would have better physiological status. All this features will increase survival rates after planting in semiarid Mediterranean conditions to fertilized seedlings compare with traditional ones.

## Materials and methods

Seedlings of *P. canariensis* C. Sm ex DC in Buch, provenance Vilaflor (FS-27/01/38/004), Tenerife, Canary Islands (Spain), were grown in commercial containers Arnabat 48C<sup>®</sup> (308 cc; 180 × 295 × 430 mm; with 48 cells per container) differing with respect to growth media and fertilizer regime. Half of the seedlings (five containers) were cultured in a 2:1 mixture of natural pine forest soil and lapilli (a volcanic gravel commonly used traditional farming within in the Canary Islands) and were kept unfertilized

(traditional method T). The other half of the seedlings (alternative method A) were cultured in a mixture 2:1 mixture of peat (Floratorf<sup>®</sup>) and lapilli, including a slow-release fertilizer (8 months) (4 g l<sup>-1</sup> of Osmocote Plus<sup>®</sup>; N/P<sub>2</sub>O<sub>5</sub>/K<sub>2</sub>O:16/8/12).

After sowing in June 2001 all the T and A seedlings cultured outdoor for 8 months in the nursery of La Laguna (600 m.). The mean daily minimum temperature of the coldest month (January) is 10°C, so growing period is continuous. In order to minimize any bias due to uncontrolled micro-environmental variations, trays of both treatments were interspersed in a completely randomised arrangement. Seedlings were watered to field capacity thrice a week by a sprinkler system. In January 2002, 25 seedlings per treatment were selected for measures of shoot height (*H*, in cm), root collar diameter (*D*, in mm), shoot dry weight (SDW; in g), root dry weight (RDW; in g), and shoot: root ratio. Ten seedlings per treatment were randomly selected for destructive sampling, dried at 60°C for 72 h and ground to 0.7 mm for determining foliar nitrogen (N), concentration according to the Kjeldahl method.

In early February 2002, 240 seedlings (24 seedlings per treatment in five completely randomized blocks) were planted at a field site near Vilaflor (28°15'28"N–16°29'40"W; 1,535 m) which is characterized by a mesomediterranean climate (Rivas-Martínez et al. 1993) with annual precipitation of 300 mm and mean annual temperature of 15°C. The growth in these area does not stop during winter, however it slightly decrease during the dry season which usually extends from June to September. According to the USDA soil taxonomy, the soil type is classified as an Aridisol. This soil type is typical for pine forests in the south of Tenerife and is characterized by a low content of organic matter and a low water holding capacity (Luis 2006). Within each block, T and A seedlings were planted randomly on a 2 × 2 m spacing scheme.

Seedling survival was assessed ten times during February 2002 throughout October 2003 while height increment was estimated six times during the period of February 2002 and February 2004.

Physiological parameters were performed in fully developed secondary needles of five plants per treatment in April, June, and August 2004. Leaf water potential ( $\Psi$ ) was measured with a Scholander pressure chamber (PSM Instruments, Corvallis, OR, USA). CO<sub>2</sub> assimilation rate (A) and stomatal conductance (g<sub>s</sub>) were determined using an ADC-LCA4 gas analyzer (ADC Systems, England). Gas exchange parameters were calculated according to von Caemmerer and Farquhar (1981) and expressed by unit of projected leaf area. Components of chlorophyll fluorescence were quantified in situ with a portable modulated fluorometer (Mini-PAM, Walz, Effelrich, Germany) equipped with a leaf-clip holder (2030-B, Walz) and a

microquantum sensor for monitoring photosynthetic photon flux density (PPFD). After clamping the leaf-clip holder onto attached needles, the actual fluorescence ( $F$ ) was monitored to ascertain that it was stable. Maximum fluorescence yield ( $F_m'$ ) was measured during a  $0.8 \text{ s}^{-1}$  saturating flash at  $6,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and exposure to natural illumination. The fraction of absorbed light utilised in electron transport is given by the PSII photochemical efficiency or yield parameter,  $\Phi_{\text{PSII}} = \Delta F / F_m'$  where  $\Delta F = F_m' - F$  (Genty et al. 1989). Non-photochemical quenching (NPQ) described by  $(F_m - F_m') / F_m'$  and photochemical quenching (qP) described by  $(F_m - F') / (F_m' - F_0)$  were also determined.

In September 2004, height, root collar diameter, shoot and root biomass, and nitrogen concentration were determined in needles in the five plants per treatment used to measure the above-mentioned physiological parameters. Root biomass was extracted by excavating down to only 70 cm depth because of the existence of a very rocky soil horizon below that threshold.

One-way ANOVA was used as the statistical tool (SPSS (v. 11.5 SPSS Inc., Chicago, IL, USA) to account for significant differences between treatments at  $P < 0.05$ .

## Results

After one culture period of 8 months in the nursery A seedlings displayed a significantly height growth, diameter, shoot: root ratio and produced bigger shoots (5.6 times more shoot biomass) and roots (2.5 times more root biomass) as compared to T seedlings (Tables 1, 2). A seedlings had also a significant higher N content (Table 2) nevertheless both T and A seedlings were within the range reported for foliar mineral nutrient concentrations of *P. canariensis* at various field sites in Tenerife (Tausz et al. 2004).

From February 2002 to August 2002 the survival rate of T and A seedlings declined to 65 and 95%, respectively (Fig. 1). After the first summer, however, this trend tailed

**Table 2** Statistical differences between traditional (T) and alternative (A) seedlings before and 3 years after planting

	Before Planting		3 Years After Planting	
	$F$	$p$	$F$	$p$
H (cm)	605.840	0.0001	56.075	0.0001
D (mm)	452.285	0.0001	29.450	0.0001
SDW (g)	431.123	0.0001	21.538	0.002
RDW (g)	244.278	0.0001	11.676	0.009
S:R	330.804	0.0001	1.445	0.264
N ( $\text{mg g}^{-1}$ )	63.85	0.0001	0.110	0.745

$H$  height,  $D$  root collar diameter,  $SDW$  stem dry weight,  $RDW$  root dry weight,  $S:R$  shoot:root ratio,  $N$  foliar nitrogen concentration

and approached 60 and 90% towards the end of the observation period in October 2003. Furthermore, throughout the whole experimental period height growth of A seedlings was superior when compared to T seedlings (Fig. 2) and after 3 years of accumulation was double for A, relative to T seedlings. After this time, the relative differences in SDW between T and A seedlings were similar to those at planting (5.3 times more SDW in A), but RDW differences increased sharply (5.5 times more RDW in A) (See also Fig. 3). Three years after planting, shoot:root ratio and nitrogen foliar concentration were similar in both treatments, and no significant differences were observed (Table 2).

During the summer of 2004, leaf water potential ( $\Psi$ ) increased from April through August in both T and A seedlings (Fig. 4). Although A seedlings always displayed a higher  $\Psi$  as compared to T seedlings, differences were only statistically significant in April and June. Foliar gas exchange by contrast declined progressively throughout the summer of 2004 (Fig. 5). This observed seasonal trend of A and  $g_s$  was pronounced in T seedlings and became statistically significant in August.

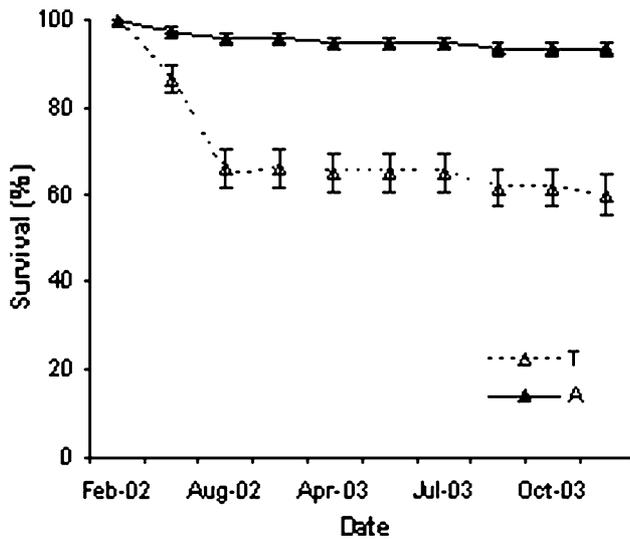
In parallel with A and  $g_s$ , fluorescence parameters also decreased through the summer (Fig. 6). Statistically within

**Table 1** Mean and standard errors of the measured parameters before and 3 years after planting for traditional (T) and alternative (A) seedlings

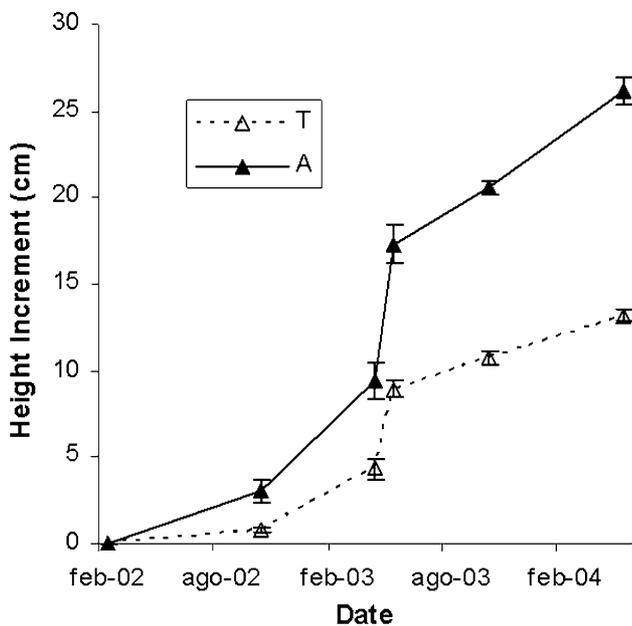
	Before planting		3 Years after planting	
	T	A	T	A
H (cm)	$8.1 \pm 0.16$	$20.1 \pm 0.34^*$	$55.8 \pm 3.86$	$100.0 \pm 4.46^*$
D (mm)	$23.4 \pm 0.03$	$40.4 \pm 0.03^*$	$191.8 \pm 21.74$	$342.0 \pm 17.46^*$
SDW (g)	$0.57 \pm 0.02$	$3.22 \pm 0.319^*$	$37.0 \pm 7.64$	$197.9 \pm 50.52^*$
RDW (g)	$0.52 \pm 0.01$	$1.28 \pm 0.128^*$	$40.7 \pm 8.64$	$215.9 \pm 50.05^*$
S:R	$1.10 \pm 0.04$	$2.53 \pm 0.11^*$	$0.38 \pm 0.028$	$0.47 \pm 0.07$
N ( $\text{mg g}^{-1}$ )	$10.0 \pm 0.11$	$21.5 \pm 0.13^*$	$10.4 \pm 1.4$	$11.9 \pm 1.2$

$H$  Height,  $D$  root collar diameter,  $SDW$  stem dry weight,  $RDW$  root dry weight,  $S:R$  shoot:root ratio,  $N$  foliar nitrogen concentration

\*Significant differences between treatments at  $P < 0.005$



**Fig. 1** Survival (%) of T- (open triangle) and A-seedlings (filled triangle) during 2 years after plantation

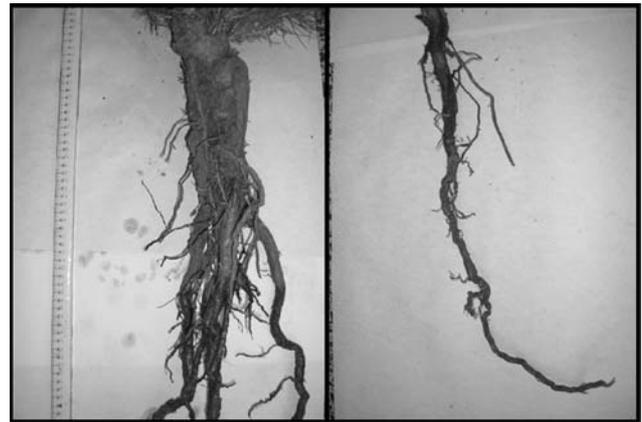


**Fig. 2** Accumulated height increment (cm) for traditional (T-seedlings, open triangle) and alternative (A-seedlings, filled triangle) between October 02 and May 04

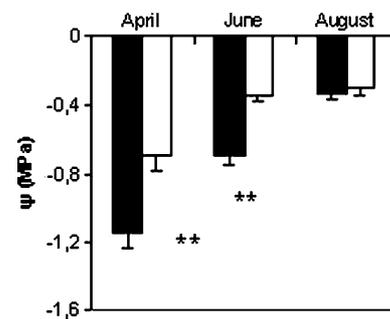
treatment effects were absent in April. In June and August,  $\Phi_{PSII}$  and  $qP$  were significantly higher in A as compared to T seedlings, while the opposite was found for NPQ. The latter, however, was not statistically significant.

## Discussion

As expected, differences in the cultivation method with respect to growth media and fertilizer regime had a signifi-



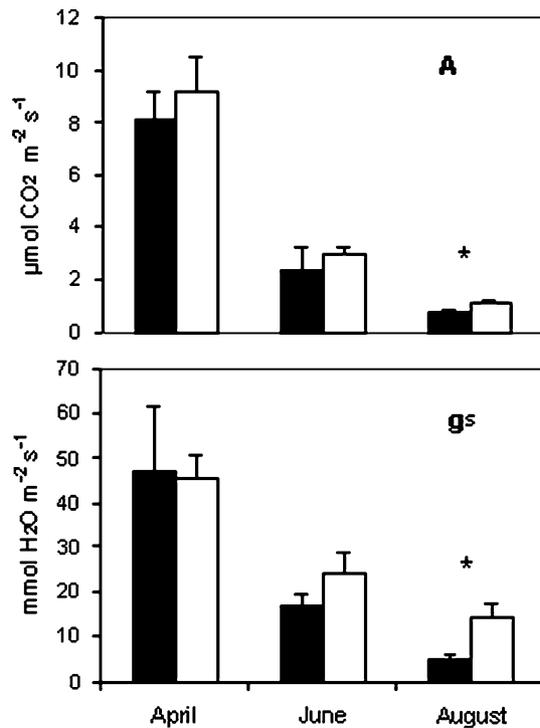
**Fig. 3** Roots from root collar to 70 cm depth into soil of A (right) and T plants (left) at the end of third year



**Fig. 4** Midday water potential ( $\psi$ ) for T-plants (black columns) and A-plants (white columns). Asterisks show significant differences between stock-types within each day (\*\* $P < 0.001$ )

cant impact on seedling features survival rate and field performance. Similar strong responses in seedling development and internal nutrient levels to differences in nutrient availability in the nursery have also been reported for other *Pinus* species (van den Driessche 1992; Fraysse and Crémière 1998; Villar-Salvador et al. 2000; Nogués and Alegre 2002; Puértolas et al. 2003).

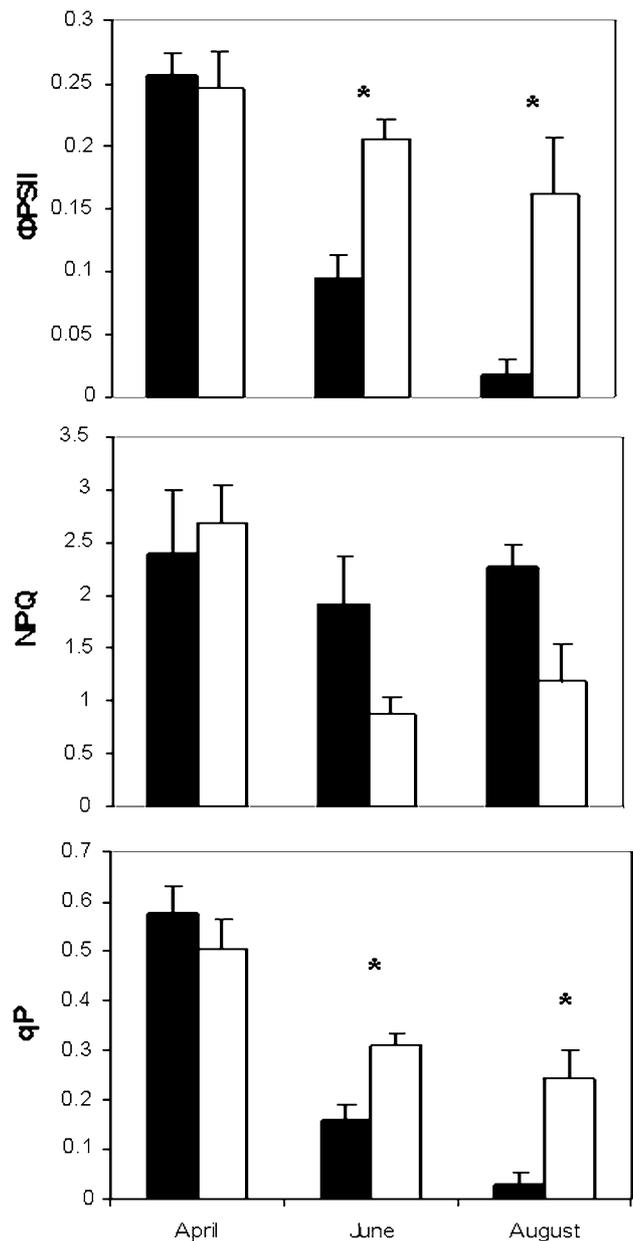
These better-quality culture conditions in the nursery were also reflected in a better field performance of A seedlings which had significantly enhanced survival rate and height growth as compared to T seedlings as also observed by others (Mexal and Landis 1990; South and Mitchell 1999). Larger seedlings generally display a greater photosynthetic active surface in terms of needle biomass. Thus, they have a higher net carbon gain which may increase the survival rate. There is evidence that the survival rate of *P. halepensis* and *P. pinaster* tends to increase with seedling size (Cortina et al. 1997; Oliet et al. 1997). Although an increasing shoot:root ratio sometimes has been reported to be negative for water stress avoidance and survival (Thomson 1985; Haase and Rose 1993), we obtained better rates of survival for the A plants with more than twofold higher shoot:root ratio than T. This suggests that low shoot:



**Fig. 5** CO<sub>2</sub> assimilation rate (A) and stomatal conductance rate (g<sub>s</sub>) for T-plants (black columns) and A-plants (white columns) at noon. Asterisk shows significant differences between stock-types (\*P < 0.05)

ratio per se is not an advantage for containerized seedlings planted in dry sites (Bernier et al. 1995; Lamhamedi et al. 1997). Interestingly, the initial large differences in shoot:root ratio reduced dramatically in both treatments 3 years after planting, reflecting the acclimation to the semiarid post-planting environment. However, this decrease was much pronounced in the improved seedlings due to the higher biomass allocation to roots compared to the T seedlings (relative differences between treatments in root biomass changed from 2.5 at planting to 5.5 3 years after, while shoot relative differences remained stable). Therefore, the much higher shoot:root ratio of improved seedlings seemed to induce a higher post-planting biomass allocation to roots, which enhanced water stress avoidance. This may be a specific behavior of *P. canariensis*, which is known to have an adaptive high biomass allocation to roots in the seedling phase (Climent et al. 2008).

On other hand, nutrient concentration in A seedlings was much higher than in T seedlings. It has been shown that increasing nutrient concentration (especially nitrogen) enhances photosynthesis rates (Jose et al. 2003; Gough and Seiler 2004). Retranslocation is also an important source of nutrients to build up the growing tissues (Boivin et al. 2002). Therefore, high nutrient concentration enhances early growth. Since fertilization usually increases not only nutrient concentration but also shoot biomass, it is difficult to disentangle their effects on early growth. However,



**Fig. 6** PSII photochemical efficiency (Φ<sub>PSII</sub>), non-photochemical quenching (NPQ), and photochemical quenching (qP) for T-plants (black columns) and A-plants (white columns) at noon. Asterisk shows significant differences between stock-types (\*P < 0.05)

evidences from late-season fertilization experiments support the idea that nutrient concentration plays an important role in early growth (Puértolas et al. 2003; Boivin et al. 2002). However, the extent of this effect is limited. Three years after planting, differences in nutrient concentration had disappeared. Early growth analyses in the Mediterranean pine *P. halepensis* suggested that initial higher nutrient concentration enhanced growth only during the first year after planting. After this time, shoot size determined growth (Puértolas et al. 2003).

The observed decline in  $A$  and  $\Phi_{\text{PSII}}$  in both T and A seedlings throughout the rainless summer of 2004 can be attributed to stomatal closure (Flexas and Medrano 2002, Flexas et al. 2002a, b; Medrano et al. 2002), one of the main processes that causes photosynthesis to decline (Chaves et al. 2002, 2003) during periods of increasing soil drought.

Stomatal conductance has been recognized as the most useful parameter to detect moderate drought stress (Medrano et al. 2002; Chaves and Oliveira 2004) as we saw in this study. On other hand, fluorescence parameters were the most discriminate between treatments. The seasonal decrease in fluorescence parameters was probably also due to the effects of soil humidity depletion leading to stomatal closure. This impairs  $\text{CO}_2$  uptake and, in turn, quantum yield is reduced (Genty et al. 1989; Loreto et al. 1995; Flexas et al. 2004). However, the impact on fluorescence parameters were exacerbated by the high radiation loads observed during the measures (c.a.  $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Nevertheless, A seedlings showed higher rates of  $\Phi_{\text{PSII}}$  and  $qP$ , indicating a better physiological status during the drought period. Analogously, non-photochemical quenching was higher in T seedlings, reflecting that these seedlings had a lower photosynthetic electron flux and needed to dissipate a higher excess of energy than A seedlings.

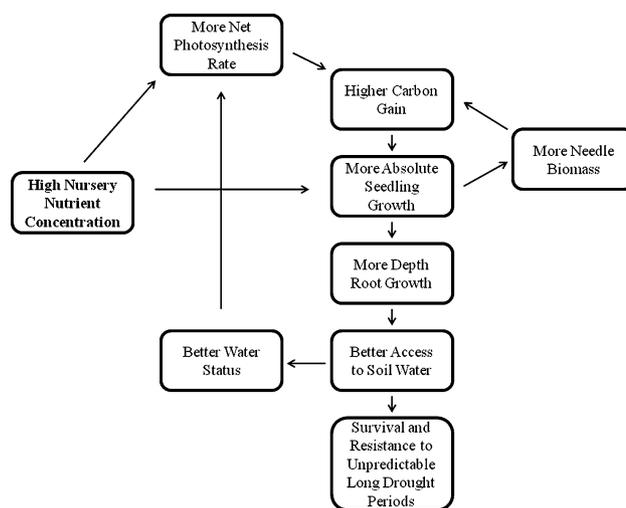
One of the most commonly used water status parameters to assess water stress intensity is leaf water potential (Flexas et al. 2004). Even 3 years after planting, when mortality rates in both treatments were close to zero, the improved seedlings had higher  $\Psi$  in early spring than traditional seedlings. This better water status in T seedlings was linked to the higher root biomass, suggesting that the higher root growth during the first years after planting improves the seedling access to water resources. Probably, the differences in root growth rate were more decisive in the wet months after planting leading to critical differences in plant water status that determined summer mortality. However, despite the lack of survival differences between treatments in the third year, the better water status of the improved seedlings indicates that these plants could better withstand an eventual period of extreme drought. These unpredictable events are typical from the semiarid environments and may cause high mortality several years after planting, when survival seems to be stable.

Surprisingly, midday water potential increased during the dry season, despite the absence of rainfall. Measurements in July and especially in August were preceded by a dense mist falling at predawn and raising 2 or 3 h before the measurement time at midday. Rehydration of shoot tissues by the mist presumably raised plant water potential. It has been demonstrated that dew or mist absorption by foliage is an important source of water uptake in dry environments, raising plant water potential (Waisel 1958; Boucher et al. 1995; Munné-Bosch et al. 1999) but does not affect the

photosynthetic machinery. Photosynthesis recovery following severe soil water stress may take several days, or even weeks, with optimal soil water availability (Harley et al. 1987; Munné-Bosch and Alegre 2000; Nogués and Alegre 2002).

Nevertheless in August,  $A$  and  $g_s$  were significantly higher in A seedlings compared to T seedlings, despite the absence of differences in  $\Psi$ , and thus confirming that A seedlings had better water status than T.

The obtained results allow proposing a conceptual model to explain containerized *P. canariensis* seedlings establishment planted in semiarid environments (Fig. 7). The proposed feed-back model relies on the assumption that pre-planting nutrient concentration (especially nitrogen concentration) is positively related to net photosynthesis rate (Green and Mitchell 1992; Grossnickle 2000; Jose et al. 2003) and subsequently, to net carbon gain. Higher needle biomass also increases net carbon gain through a higher photosynthetic surface. Enhanced carbon gain increases root biomass and length, which allows the seedling exploring greater and deeper soil areas, determining its survival probability (Padilla and Pugnaire 2007). This better access to water can enhance not only plant survival during long drought periods but also photosynthesis during the onset of drought. The observed progressive increase in the differences of seedling height between A and T seedlings during the 3 years of study, and the strong relationship between root biomass and water status in the third year confirms the feedback model. This conceptual model is inspired in that proposed by Burdett (1990) for bare-root conifer seedlings planted in boreal and temperate sites. However, some differences between plantation in humid and semiarid zones must be taken into account. First, Burdett's model emphasizes the importance of the



**Fig. 7** Feed-back model for *Pinus canariensis* establishment in a semiarid environment

establishment of soil-root contact immediately after planting in order to decrease the resistance to water flow through the soil-air-plant-continuum. Therefore, the very early root growth, which extends several days or weeks, is the most decisive phase for seedling survival. Thus, the feedback model for humid climates operates in the short term. Once the roots have contacted with the soil, the system becomes stable, as there is not much water depletion in the upper soil layers. Containerized seedlings can have lower resistance to water flow through the soil-plant-air-continuum after planting than bare root seedlings (Grossnickle 2005). In this situation, early root growth is not limited if seedlings are planted properly (granting soil-plug contact) and there are no functional problems in the roots provoked by frosts, diseases or bad handling (McKay 1997). Therefore, if early root growth and, in turn, soil-root contact, is granted by using healthy plants and adequate handling and planting practices, the decisive phase for containerized seedlings is the wet period after planting, which extends several months after planting, when a rapid and sustained root growth is desirable. Second, climate in temperate and boreal zones is much more predictable than in semiarid environments where extremely long droughts can occur. Therefore, plantations that seem to be well established can fail during one of these events. Therefore, the feedback model for Mediterranean and semiarid climates operates in the mid and the long term. It is desirable that seedling root will be quick and sustained during the first years after planting to escape from soil water depletion in the upper soil layers. The deeper the roots, the longer the drought period can be withstand. Our results show that planting rapid-growing *P. canariensis* seedlings not only reduces the risk of mortality in the short term but also increases the resistance of the plantations to drought in the long term. Even 3 years after planting, water status was better in seedlings which developed the larger and deeper root system.

However, there are some concerns in this conceptual model that deserve further research, specially for understanding the establishment of other species in the semiarid climates. This model ignore that higher needle biomass implies more water consumption. Fertilization is not always beneficial, since an excess of fertilization lead to higher water losses and an imbalance in the shoot to root ratio. In a water-saver species like *P. canariensis* (Morales et al. 1999) this is a minor problem, since it has a good stomatal control of water losses. However, in very harsh sites where root growth is difficult or when planting water-spending species, the higher water consumption by the improved seedlings could impair survival. This could be the explanation of the results of studies performed in semiarid climate that observed a better field performance for nutrient deprived seedlings (Trubat et al. 2008).

We conclude that culture media and fertilizer regime in the nursery significantly affects seedling size. As peat and added fertilizer significantly increased seedling growth and thus significantly contributed to the survival and the overall field performance of *P. canariensis* seedlings under field conditions we recommend the use of well fertilized, *P. canariensis* seedlings for facilitating reforestation in semiarid sites within the Canarian Archipelago. The results suggest that *P. canariensis* establishment can be explained by a conceptual feed-back model, with high nutrient concentration and large needle biomass improving field growth and water status which, in turn, enhances subsequent growth and survival.

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