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6 **Root structure and biomass partitioning in tilted plants from twisted- and straight-**
7 **stemmed populations of *Pinus pinaster* Ait.**

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12 Abstract

13 *Pinus pinaster* Ait. has a tendency to exhibit stem flexuosity that negatively affects the quality of its wood
14 and its productivity. There is a wide geographical variability in this trait, and there is evidence of genetic
15 control. We hypothesized that root structure and biomass allocation adjustments in response to a given
16 mechanical stress might differ among populations of *P. pinaster* and might be related to the typical
17 straightness of the stems of a given population. We analyzed root structure and biomass allocation in a
18 provenance test in which plants were artificially tilted at 45° and naturally exposed to wind. Ten
19 provenances were tested: five with typically straight-stemmed plants and five with twisted-stemmed
20 plants. The wind affected the taper and the development of thickenings in the windward second-order
21 roots, although the winds experienced were generally light. The straight-stemmed populations exhibited
22 greater variability in the studied traits than the twisted-stemmed populations. This variability may reflect
23 higher root responsiveness as well as various strategies to address mechanical stresses. Three possible
24 additional distinguishing characteristics of various straight-stemmed populations are proposed: a) greater
25 allocation of biomass to the stem compared with the branches, b) development of a thick, cylindrical

26 taproot and tapered lateral roots and c) strengthening of second-order roots with local thickening in the
27 sectors of the root under tension.

28

29 Keywords: roots, biomass, biomechanics, provenance test, stem straightness, wind.

30 Key message: Straight-stemmed populations of *Pinus pinaster* under mechanical stress allocate more
31 biomass to the stem relative to the branches and show greater variability in the roots than twisted-
32 stemmed populations.

33 Author Contribution Statement:

34 Fermín Garrido: performed field and lab work, analysis and discussion of the results in collaboration with
35 the other authors.

36 Roberto San Martín: performed the statistical analysis and collaborated in the discussion of the results.

37 Francisco José Lario: collaborated in establishing hypotheses, designing the structures of the trials, took
38 over the plants in the experiment and assisted in the field work.

39 Rosario Sierra-de-Grado: conceived the research project, obtained the funding, collaborated in analysis
40 and discussion of the results and wrote the paper.

41

42 Conflict of Interest: The authors declare that they have no conflicts of interest.

43

44 **INTRODUCTION**

45 *Pinus pinaster* Ait. is one of the main forest species with respect to range and wood production in several
46 countries. This species' tendency to exhibit a lack of stem straightness is of considerable concern and
47 affects its quality and economic profitability. Tree stem flexuosity shows large natural variability in *P.*
48 *pinaster* populations from different geographic regions (provenances). In provenance common garden
49 tests established in different environments, the typical stem straightness of the tested populations is
50 usually stable and maintains the typical stem form (straight or twisted) representative of the population in
51 the test plants' places of origin, demonstrating genetic control of this trait (Alía et al. 1995; Sierra-de-
52 Grado et al.1999).

53 The economic importance of stem straightness has led to include it as a selection trait in most
54 genetic improvement programs for this species. The heritability values for stem straightness in *P. pinaster*
55 are very variable and range from low (0.03 ± 0.02 , Coterill et al. 1987) to high values (0.9, familial
56 heritability in Magini 1969). This variability may be partially caused by different definitions of the trait
57 (from angles to subjective evaluations, including the presence of forking or other defects) and
58 methodological limitations in the quantification of stem form in which the current form of the stem is
59 focused on while factors and processes that cause stem defects, which are often stochastic, and processes
60 of postural control in the plants are ignored.

61 A lack of straightness is frequently associated with tree stability and wind. The inclination of the
62 trees in reforested areas after an episode of strong winds (toppling) subsequently causes curvature in the
63 base of the trunk, which leads to an important loss of economic value (Coxe et al. 2005). In *P. pinaster*,
64 toppling problems usually occur when the trees reach a height of approximately 90 cm, during the first 2
65 to 5 years after planting (Ocaña et al. 2001; Lario and Ocaña 2004). Crémère (2003) studied the causes
66 of instability in conifer plantations, indicating root quality and genotype as primary factors, among others.
67 Toral et al. (2011) found a correlation between the quality of the taproot and the probability of toppling in
68 *P. radiata* plantations. A relationship between stem straightness and anchorage has also been noted by
69 Danjon et al. (1999), who considered a low shoot: root ratio and a high proportion of deep roots to be
70 promoters of straightness in *P. pinaster*. Important acclimation processes also occur when the trees
71 experience mechanical stimuli, such as wind, which affect the root system and, in turn, stability (Danjon
72 et al. 2005, Coutand et al. 2008).

73 Many environmental factors can affect stem form (wind, landslides, snow, phototropism, etc.);
74 however, a process of stem straightening occurs whenever the stem is tilted or bent to restore a
75 mechanically stable position (Moulija et al. 2006). The efficiency of the stem straightening process
76 exhibits genetic variability at the population level and has been related to the typical stem straightness of
77 the population (Sierra-de-Grado et al. 2008). Detailed biomechanical studies have been conducted on
78 artificially inclined *P. pinaster* seedlings (Loup et al. 1991; Fournier et al. 1994), and both the kinetics
79 and the gravitropic and autotropic components of the stem straightening process after tilting are well
80 known. Sierra de Grado et al. (2008) suggested that the straightest populations could have a greater
81 capacity for stem straightening than plants from the sinuous provenances, mainly because of a more
82 efficient reaction wood (i.e., higher maturation strains). However, the impact of the process of
83 straightening on the roots and biomass allocation remains largely unknown.

84 In young trees, the taproot plays an important role as the distribution axis for lateral roots and
85 as an anchorage system with which to resist strong winds (Balneaves and De La Mare 1989; Auberlinder
86 1982; Burdett et al. 1986; Crook et al. 1997; Crook and Ennos 1998; South et al. 2001). Several authors
87 have described the “inherently regular” architectural pattern of the roots in conifers (Khuder et al. 2007 in
88 *P. pinaster*, Henderson et al. 1983 in Sitka spruce). In *P. pinaster* seedlings and cuttings, if the taproot is
89 removed, either it re-develops or a lateral root assumes its role (Khuder et al. 2007). However, the role of
90 the taproot in the stability of trees appears to be of secondary importance in large trees or in shallow soils
91 (Ennos 2000; Peltola 2006; Danjon et al. 2005) in comparison to the stabilizing function of the shallower
92 lateral roots and the root-soil plate.

93 The development of a root system with an even lateral distribution allows plants to obtain
94 mechanical stability (Danjon et al. 2005, Fourcaud et al. 2008). The development of plants is limited if
95 root growth is restricted, causing a reduction in leaf area, height and biomass production (Reis et al. 1989;
96 Townend and Dickinson 1995), although different methods of imposing such restrictions (limitation in
97 depth or in lateral extension) have different effects on growth responses (Korndorfer et al. 2008). Many
98 forest soils, including soils where *P. pinaster* grows, have a shallow depth. Rocks or flooding areas may
99 also limit the lateral extension of the roots. Given the need for good anchorage, the root system must be
100 able to adjust to the limitations imposed by the soil and develop the most efficient structure according to
101 the particular conditions at each site (Danjon et al. 2005, Danjon et al. 2013). Therefore, trade-offs can be
102 expected between size variables (length, diameter) and shape variables (taper, winding, branching) of the

103 taproot and second-order roots to form the most effective root system for the particular conditions of each
104 site.

105 Mechanical stresses such as wind are known to increase root development (Cucchi et al. 2004;
106 Richter et al. 2009, Danjon et al. 2013), most likely at the expense of the above-ground part of the plant,
107 which is seriously limited by the action of the wind, even if the wind is not of high intensity (Mouliat et al.
108 2011). Artificial flexing of low intensity also results in major changes in the structure of stem and roots
109 (Danjon et al. 2013). Coutand et al. (2008) found that mechanical stimuli regulated the shoot: root ratio
110 and that movements such as those induced by wind sway increased biomass allocation to the roots. This
111 response can be explained according to the functional equilibrium theory; plants respond to stressful
112 environmental conditions, including mechanical stresses such as wind or tilting, by shifting carbon
113 allocation to the more efficient organs to reduce the stress (Mäkelä 1999; Chambel et al. 2007; Coutand et
114 al. 2008). Roots are very sensitive and react rapidly to above-ground events. Moreira et al. 2012 reported
115 rapid changes in biomass and nutrient allocation in response to simulated herbivory in *P. pinaster*. In
116 response to the signal of herbivory damage, seedlings showed a strong increase in the fine root system,
117 whereas the growth of coarse roots and above-ground structures was reduced. The roots are also sensitive
118 to wind and acclimate to the different types of stress that wind imposes (i.e., tension, compression and
119 torsion), for example, by modifying the size and shape of the cross section to increase its strength
120 (Nicoll and Ray 1996; Stokes and Mattheck 1996).

121 We hypothesize that root structure and biomass allocation adjustments in response to a given
122 mechanical stress may differ among populations in *P. pinaster*. Taking into account the large genetic
123 variability shown by *P. pinaster* in most of the quantitative traits and molecular markers studied (Alía et
124 al. 1997; Salvador et al. 2000; González-Martínez et al. 2005; Chambel et al. 2007), including root
125 morphology (Corcuera et al. 2012) and changes in biomass partitioning as a drought tolerance mechanism
126 (Aranda et al. 2010), we presume that some variability might exist in root traits and in their anchorage
127 efficiency. This variability may be related to the typical stem straightness of the populations in their
128 environments of origin and in provenance trials, as in both cases, trees are subjected to mechanical
129 stresses (wind, snow, etc.).

130 We have analyzed roots and biomass allocation in a provenance test, in which plants were
131 artificially tilted and naturally exposed to wind. In the present study, our goal was to investigate whether
132 different populations of *P. pinaster* subjected to mechanical stress (artificial tilting and the wind

133 conditions of the experimental site) showed differences in root structure and biomass partitioning and, if
134 so, whether the typical stem straightness of the populations is related to those traits.

135

136 **MATERIALS AND METHODS**

137 *Plant material and experimental procedures*

138 Ten seed sources were used, five of them from typically straight-stemmed provenances and five
139 from typically twisted-stemmed provenances, according to the straightness classification based on data
140 from five provenance trial sites in Spain (Alia et al. 1995). Hereafter, we refer to the populations using the
141 acronyms defined in Table 1.

142 Table 1

143 The experiment was performed in the nursery of the Tragsa Company in Maceda (Ourense),
144 where the prevailing winds during the late spring and summer come from the west. The plants were
145 sowed on July 16, 2008, in round pots 30 cm in diameter and 30 cm deep. An 80:20 mixture of peat and
146 perlite was used as the substratum. Germination and the first growing season were conducted under a
147 shade cloth. In late May 2009, the plants were placed outdoors and distributed on a frame of 3x3 m
148 according to a randomized complete block design with 10 blocks and one plant per provenance and block.
149 Under the shade cloth, the plants were irrigated by sprinkling, and outdoors, they were irrigated by
150 dripping. The plants were fertilized with Osmocote® Exact® standard NPK 15-9-11-2.5 MgO.

151 Between June 11 and June 17 2009, the pots were tilted at 45° from the vertical, pointing
152 toward the south, on iron structures specially designed for the experiment (see Sierra-de-Grado et al.,
153 2008, for details on the choice of the 45° inclination). We chose those dates because differences in the
154 gravitropic reaction of the shoots were also examined (results not shown in this paper), and this reaction
155 required active growth in the shoots. At the moment of tilting, shoots had an average length of 12.5 cm.
156 The stems were allowed to grow freely while the pots remained tilted throughout the experiment. In this
157 way, we expected the main plane of the straightening reactions (N-S) to be perpendicular to the prevailing
158 winds during the experiment. At the end of October 2009, we began to extract the plants block by block
159 to be processed immediately. The last block was extracted in February 2010 so that all samples were
160 taken during the dormancy period. Before the extraction, the stem bases and root collars were marked
161 with different colors on the north and west sides to indicate the orientation of the plant parts during
162 subsequent analyses. The aerial part of the plants was separated from the root, and the roots were cleaned

163 to remove the substrate. The branches were separated from the stem. Root structure measurements were
164 performed on the taproot and the coarse second-order roots that developed without reaching the wall of
165 the container (i.e., 25 cm deep and in a radius of 10 cm around the main axis of the taproot). We defined
166 coarse second-order roots as those with a 2 mm diameter or greater at the insertion point.

167 *Measured variables*

168 We measured the N-S and E-W diameter of the taproot at depths of 0 (root collar), 10 and 25
169 cm (DTR0, DTR10 and DTR25, respectively, which indicate the mean of the two perpendicular
170 diameters) for each plant (Table 2). From these values, we calculated DTR10 and DTR25 as a percentage
171 of DTR0 (pDTR10 and pDTR25). We obtained the cross-sectional eccentricity as the ratio between the E-
172 W/N-S diameters at each observed depth (EC, EC10, and EC25). The taper index of the taproot (TTR)
173 was calculated as the ratio between the longitudinal area of the taproot and a rectangle of DTR0 base and
174 25 cm height (Fig. 1). The longitudinal area of the taproot was defined as the sum of the areas of the
175 trapezoids with DTR0 and DTR10 bases and 10 cm height and DTR10 and DTR25 bases and 15 cm
176 height. If TTR=1, the longitudinal section of the root is rectangular; TTR<1 indicates a tapered root, and
177 the more tapered the root, the lower the index value.

178 Table 2 Fig. 1

179 In each plant, we calculated the number of coarse second-order roots (NSR) with a diameter at
180 the insertion point larger than 2 mm and between 0 and 25 cm depth in the N, E, S and W sectors of the
181 root (Fig. 2a). Two perpendicular diameters at the insertion point (DSR0) and at 10 cm from the insertion
182 (DSR10) were measured in the shallowest ten coarse second-order roots; if there were fewer than 10
183 second-order roots between 0 and 10 cm in depth, we measured all of those roots. We calculated the
184 standardized values of these diameters as the percentage of DTR0 (pDSR0 and pDSR10). Using DSR0
185 and DSR10, a taper index of the second-order roots (TSR) was calculated similarly to the TTR.

186 Some second-order roots showed remarkably thickened segments. These thickenings consisted
187 of wood without signs of disease or insect attack (Julio Díez Casero, com. pers.). Some of these areas
188 exhibited bolt shapes (Fig. 2b). We recorded the presence of these thickenings in each second-order root
189 (THICK).

190 In each plant, we measured the dry weight of the following fractions: in the subterranean part,
191 coarse roots (taproot and second-order roots thicker than 2 mm in diameter, between 0 and 25 cm deep
192 and in a radius of 10 cm around the main axis of the taproot) (CRDW) and the fine roots (roots not

193 included in the previous class) (FRDW). In the aerial part, we measured the dry weight of the stem
194 (SDW) and branches (BDW), including the respective foliar biomasses. Both aerial parts and root
195 partitions were dried in an oven at 72 °C for 48 hours before weighing. Based on these data, we
196 calculated the ratio of aboveground dry weight/belowground dry weight (AGDW/BGDW) and the
197 different fractions as the percentage of the total plant biomass (BDW2, SDW2, CRDW2, FRDW2).

198 (Fig. 2)

199 *Wind*

200 Wind speed and direction were registered automatically in the meteorological station of the
201 nursery at 8-minute intervals. During the warmer months of the experiment (June to September 2009,
202 coincident with the main growing season and when all of the plants were placed at the experimental site),
203 the prevailing winds blew from the W-NW, and during the colder months (October 2009 to February
204 2010), the prevailing winds blew from the S (Fig. 3). During the warmer months, the average wind speed
205 was 5.6 m/s, reaching a maximum of 28.8 m/s, and over the course of the experiment, the average wind
206 speed was 5.8 m/s and the maximum 41.7 m/s.

207 (Fig. 3)

208

209 *Data analysis*

210 The influence of provenance and block factors on the taproot diameters (DTR0, DTR10,
211 DTR25, pDTR10 and pDTR25), cross-sectional eccentricity (EC0, EC10 and EC25) and taper (TTR)
212 were studied by adjusted analysis of variances using the PROC MIXED model. Block was considered a
213 random factor. Errors were normally distributed and independent. Restricted maximum likelihood
214 (REML) variances were calculated for each provenance. To check for significant differences between
215 straight and twisted provenances with regard to the diameter, eccentricity and taper of the taproot,
216 orthogonal contrasts were performed.

217 Data for coarse second-order root variables (DSR0, DSR10, TSR, pDSR0 and PDSR10) were
218 analyzed with a repeated-measures ANOVA according to a PROC MIXED model, where the repeated
219 measures represented the sector. Block was considered a random factor. Errors were normally distributed,
220 with different variances for each provenance and sector, independent for different roots and with constant
221 covariance for different sectors within the root. To study NSR as a function of provenance, distribution by
222 sector and the presence of THICK, a log linear model was fitted by PROC CATMOD.

223 Differences between provenances in the AGDW/BGDW ratio were analyzed with a mixed-
224 model ANOVA with provenance and block as random factors. Errors were independent and normally
225 distributed, and REML variances were calculated for each provenance. The biomass partitioning was
226 studied using a repeated-measures ANOVA with a PROC MIXED model, where the repeated measures
227 represent the part of the plant (branches, stem, coarse roots and fine roots). Errors were normally
228 distributed, with different variances for each provenance and part, independent for different trees and with
229 constant covariance for different parts within the tree. We performed a principal component analysis
230 (PCA) and a cluster analysis on all variables that showed significant differences between provenances,
231 using squared Euclidean distances and Ward's grouping method. The data were individual values
232 (n=100).

233 All the analyses were conducted with the Statistical Analysis System (SAS) software, version
234 9.2 for Windows (SAS Institute Inc., Cary, North Carolina, USA).

235 **RESULTS**

236 *Taproot*

237 The taproot diameter significantly decreased with depth in all populations (Fig. 4a). There were
238 significant differences among populations in the taproot diameter at different depths, pDTR10 and
239 tapering index (Table 3), whereas differences were not observed in pDTR25. The five twisted populations
240 (on the left side of Fig. 4) showed a great similarity in diameters (both in absolute and relative value),
241 whereas the straight populations (on the right side) showed greater variability. In absolute value, the
242 09GRE and 07NOINT provenances showed the greatest diameters at all depths, whereas 08BUSO was
243 the provenance with the minimum diameter. As a percentage of DTR0, the 10LEIR and 07NOINT
244 provenances reached the highest values of pDTR10 and 09GRE and the 08BUSO provenance reached the
245 lowest. The difference in diameter between the straight and twisted provenances was not significant at
246 any of the measured depths (Table 3).

247 Among the straight populations, 07NOINT and 10LEIR had the most cylindrical taproots (Fig. 5), with
248 taper index values exceeding 0.7, and 09GRE had the most tapered taproot, with a taper index value of
249 0.62. Based on the relative values with respect to DTR0, pDTR10 and pDTR25, the variability of the
250 shallow taproot at 0-10 cm was higher than that from the surface to 25 cm, and the 10LEIR and
251 07NOINT populations presented shallow segments of the taproot that were less tapered.

252 The asymmetry in diameter was significant at the root collar, with the N-S diameter predominantly
253 greater than the W-E diameter. There was no asymmetry at the 10 and 25 cm depths (Fig. 6). There was
254 no variation among provenances in the ratios between W-E diameter and N-S diameter (Table 3).

255

256 (Figs. 4, 5 and 6). Table 3

257 *Second-order roots*

258 The diameter of the coarse second-order roots exhibited significant differences among
259 provenances both at the insertion point and 10 cm away from it (Table 4). The average values of DSR0
260 were larger than those of DSR10, although in 4.1% of the second-order roots, the opposite was true.
261 07NOINT and 09GRE were the provenances with larger DSR0, and they also had larger taproot
262 diameters (Fig. 4b). Differences in DSR0 were significant between sectors. Second-order roots were
263 thinner at the insertion point in the northern sector than in the other sectors, although this difference was
264 due mainly to the twisted provenances (Fig. 7). Provenance and sector were both significant factors for
265 DSR10 but without any appreciable effect between the straight and twisted provenances (Table 4).

266 Provenance and sector were also significant for the taper of second-order roots, but not the
267 interaction provenance x sector (Table 4). The provenances 10LEIR and 07NOINT had more conical
268 second-order roots and more cylindrical taproots (Fig. 5). Second-order roots were more conical in the
269 eastern sector, followed by the southern, and more cylindrical in the northern and western sectors (Fig. 8).
270 The second-order roots in straight provenances were significantly more conical, overall, than in twisted
271 provenances, although the variability of TSR in the straight provenances was high (Fig. 5, Table 4). A
272 more detailed analysis showed that the differences in TSR between straight and twisted provenances were
273 caused by the lower values of TSR in straight provenances in the northern ($p=0.0064$) and eastern
274 ($p=0.0391$) sectors (Fig. 8).

275 There were no significant differences in pDSR0 between provenances or between sectors;
276 however, differences were observed in pDSR10, with the provenances 07NOINT, 09GRE and 10LEIR
277 exhibiting a lower proportion of DSR10 relative to DTR0 (Table 4, Fig. 4c).

278 (Fig. 8)

279 Table 4

280 *Distribution of coarse second-order roots and thickened segments*

281 The mean number of coarse second-order roots per plant ranged from 16.1 in 10LEIR to 20.5
282 in 02NIEV provenance. The mean distribution of NSR per sector was 4.4 roots per plant in the North
283 (23.5%), 4.5 in the East (23.8%), 4.8 in the West (25.7%) and 5.0 in the South (27%). Those differences
284 among sectors were significant, although small (Table 5, Fig. 9).

285

286 Table 5, Fig. 9

287 Remarkably thickened segments (THICK) were found in 48% of the plants and in 11.5% of
288 the second-order roots. The significant interactions Provenance x THICK (p-value=0.0212) and Sector x
289 THICK (p-value<0.0001) indicate an association between provenance and the presence of thickened
290 segments and between the sector where the roots were located and the presence of thickened segments
291 (Table 5). The provenances with a greater number of thickened segments were 06ALMO and 07NOINT,
292 with 9.9% and 8.2% of roots with thickened segments, respectively, and the lowest numbers were
293 exhibited by 04ALMI and 09GRE, at 1.05% and 3.4%, respectively. Regarding orientation, the southern
294 sector showed the minimum percentage of roots with thickened segments (1.78%), with the eastern
295 (3.59%), northern (6.37%) and western sectors (8.33%) showing higher percentages (Fig. 9).

296 ***Biomass partitioning***

297 There were differences in biomass among provenances (p-value 0.0008), parts (stem, branches,
298 coarse roots and fine roots) (p-value <.0001), and provenance x part (p-value<.0001). 09GRE was the
299 provenance with the most biomass at both aerial and subterranean levels, and 08BUSO showed the lowest
300 values (Fig. 10a). Values of AGDW/BGDW ranged between 2.06 (01ONA) and 2.65 (04ALMI). There
301 were significant differences among the provenances in AGDW/BGDW (p-value 0.012) but not between
302 the straight and twisted provenances. Aerial biomass represented, on average, 70% of the total biomass.
303 The branch biomass fraction was significantly greater than that of the stem in all of the provenances
304 except 10LEIR and 09GRE, in which the differences were not significant. Similarly, the fine root biomass
305 fraction was significantly greater than that of coarse roots in all provenances but 10LEIR, in which there
306 were no differences (Figs. 10a and 10b). There were no differences between straight and twisted
307 provenances in either the total biomass or aboveground and underground biomass, but the partitioning of
308 the aerial biomass was distinct: straight provenances showed more stem biomass and less branch biomass
309 than twisted provenances (Table 6, Fig. 10b). In absolute values, plants from straight provenances showed

310 average accumulations in the stem that were 8.71 g higher and branch biomass that was 7.99g lower than
311 that of plants from twisted provenances, with differences of +6.59% in stems and -6.47% in branches.

312 Table 6 Fig. 10.

313 *Correlations between variables at the individual level*

314 In general, all of the diameters of taproot and second-order roots were well correlated (Table 7).
315 There was a moderate correlation between stem and branch biomass ($r=0.447^{***}$) and between coarse
316 and fine roots biomass ($r=0.518^{***}$). Whereas coarse root biomass was well correlated with both branch
317 and stem biomass, fine root biomass showed a stronger correlation with branch ($r=0.652^{***}$) than with
318 stem biomass ($r=0.346^{***}$). In addition, there was a strong and negative correlation ($r=-0.705^{***}$)
319 between the stem and branch biomass proportion relative to the total plant biomass (BDW2 and SDW2).
320 The tapers of taproots and second-order roots were slightly and negatively correlated and showed no
321 correlation with the biomass.

322 Table 7

323 *PCA and Ward's cluster analysis*

324 At this point in the analysis, only three of the orthogonal contrasts between straight and
325 twisted provenances applied to variables individually were significant: the taper of second-order roots
326 (TSR was larger in straight provenances), the DSR0 of the roots in the northern sector (larger in straight
327 provenances) and the difference in allocation of aerial biomass between the branches and stems (stem
328 biomass is larger in straight than in twisted provenances, whereas branch biomass is smaller). The PCA
329 provides information on the global performance of the provenances accounting for all significant
330 variables together (Fig. 11) and the associations among variables. The three first components (F1, F2 and
331 F3) of the PCA accounted for 66.11% of the variance (39.76, 16.27 and 10.08%, respectively). F1 was
332 positively correlated with the variables of plant size; F2 was positively and strongly associated with TTR
333 and negatively correlated with TSR, and F3 showed multiple correlations with heterogeneous variables,
334 including the number of thickened segments. Notably, straight-stemmed populations showed greater
335 heterogeneity than twisted-stemmed populations and more extreme coordinates in the PCA axes.

336 This situation was reflected in the cluster analysis (Fig. 12), where all of the twisted
337 populations could be grouped together in the most homogeneous group of association, and the straight
338 populations could be associated in three groups, including one mono-provenance group (09GRE) and two
339 groups containing two populations each (06ALMO with 08BUSO and 10LEI with 07NOINT).

340 **Fig. 11 and 12**

341

342 **DISCUSSION**

343 *Effects of tilting and wind on roots*

344 The observed differences in sectors (N, S, E, W) may be associated with mechanical effects
345 acting in different directions: the artificial tilting of the plant to the S, which implies compression and
346 bending in the S sector (downward) and tension in the N (upward), and the dominant wind from the W-
347 NW during the warmer months, which induces compression and bending in the E sector (leeward) and
348 tension in the W (windward), with a component of repetition and alternation due to swaying that is not
349 present with tilting. The similarities between the roots of the N and W sectors in contrast to those of the E
350 and S sectors (i.e., second-order roots in the N and W sectors are less tapered and have a greater number
351 of thickened segments than those in the E and S sectors) are consistent with the distribution of tension and
352 compression stresses. This finding suggests that wind affected the measured variables mainly during the
353 warmer months, essentially during the growth period, whereas the wind during the colder months
354 (prevailing from the S) had no effect or an effect that was canceled out by the more important effect of
355 tilting. Nicoll and Dunn (2000) found little influence of wind on root development during the main shoot
356 growth season in a 46-year-old wind-exposed *Picea sitchensis* plantation in the UK, but the milder
357 climate and the nursery conditions that enhanced growth (fertilization and irrigation) in our experiment
358 made a comparison with Nicoll and Dunn's findings difficult.

359 The cross section of the taproot was elliptical at the collar level, with the N-S diameter larger
360 than the E-W diameter in most plants. This asymmetry was lost in the deeper cross sections of the root.
361 These findings suggest that the asymmetry at the root collar results from the asymmetric growth of the
362 stem due to tilting and that the root response is independent from that of the stem. In stems, a remarkable
363 cross-sectional asymmetry in tilting experiments has been found (Loup et al 1991; Fournier et al. 1994;
364 Sierra de Grado et al. 2008), and stems in the present experiment also developed severe asymmetry, with
365 a larger southern radius along the curved stem (data not shown). The collar cross sections also showed
366 larger southern radius. The wind appears to have no effect on the asymmetric growth of the collar cross
367 section or on the deeper cross sections of the taproot.

368 Roots in the W sector were less tapered and had a greater number of thickened segments than
369 those in the E sector, which suggests a greater allocation of root biomass in the windward sector. The

370 differences between the N and S sectors were not particularly strong, although there were more thickened
371 segments in roots from the N than those from the S, and the DSR0 was smaller in the N. It is noteworthy
372 that in the studied root variables, wind appears to have a stronger effect than a 45° inclination, even
373 though the winds experienced were generally light. Danjon et al. (2013) found that flexed trees growing
374 on a 45° slope developed more volume in the upslope surface lateral roots, whereas there were no
375 differences if the trees were not flexed.

376 *Local thickening as an alternative to length growth*

377 Apart from the ordinary diameter variations in second-order roots, we observed notable local
378 thickening that may play a role in anchorage. These thickened segments appeared mostly in the W and N
379 sectors, modifying the longitudinal and cross-sectional shape of the roots and presumably their
380 mechanical properties. The substantial increase in cross-sectional area resulting from these thickened
381 segments may act as a reinforcement to prevent root failure or uprooting due to wind or tilt. The lateral
382 area of the roots with this type of thickening also increases, and consequently, the friction soil-root also
383 increases. It is known that lateral roots exhibit asymmetrical secondary growth, developing T- and I-beam
384 cross-section shapes to better resist imposed bending stresses (Cannell and Coutts 1988; Stokes et al
385 1998). The observed thickenings combine changes in shape with an increase in cross section and can thus
386 be very efficient at improving stiffness and producing a large friction surface.

387 In plants grown in pots, where the development of long second-order roots to improve resistance
388 to tension is prevented, local thickening may be an alternative. This process might also occur under field
389 conditions when the roots encounter impenetrable obstacles. We have observed similar local thickening in
390 the roots of several species grown in field conditions, but to our knowledge, this phenomenon has not
391 received attention until now.

392 *Variability between twisted- and straight-stemmed populations*

393 Straight provenances showed greater heterogeneity than twisted provenances in almost all the
394 analyzed variables. This pattern was also reflected in the PCA. All twisted provenances were quite similar
395 in the variables measured, whereas straight populations combined the traits of size and shape of the roots
396 (DTRs, DSR0, TTR and TSR) and biomass partitions in a more variable way than the twisted populations
397 and exhibited more extreme values. This pattern suggests a higher responsiveness in straight-stemmed
398 populations when they encounter mechanical stresses and a more effective modulation of the shape and
399 size of their roots and biomass allocation, although each population exhibited a different strategy.

400 Different intraspecific strategies have been described for other character sets in *Pinus* species by Tapias et
401 al. (2004) and, in *P. pinaster* specifically, by Nguyen and Lamant (1989) and Aranda et al. (2010), who
402 described different population strategies in response to drought in which roots were involved. In our case,
403 to verify that these differences are due to a response to mechanical stress and not an intrinsic
404 characteristic of the roots of each population, a comparison between mechanically stressed and unstressed
405 plants would be required, but the idea that straight-stemmed populations are able to respond more
406 efficiently than twisted-stemmed populations is consistent with the present study.

407 In a first approach, only the taper of coarse second-order roots and aerial biomass partitioning
408 (between stem and branches) showed significant contrasts associated with the typical straightness of the
409 populations. However, plants respond to the environment in an integrated manner; thus, a more
410 integrative analysis can provide meaningful information that goes unnoticed when studying isolated
411 variables. Correlations between variables and ACP are useful tools for such analyses.

412 In straight provenances, second-order roots were significantly more tapered than in twisted
413 provenances, although they showed great variability. The contrast between straight and twisted
414 provenances was not significant for TTR, but two of the straight populations (NOINT and LEIR) that
415 showed very cylindrical taproots were also the populations with more tapered second-order roots. These
416 two populations also showed a higher pDTR10. This finding suggests that NOINT and LEIR develop
417 more cylindrical taproots and reinforce the shallower portion of the taproot, perhaps at the expense of the
418 second-order roots. The taproots in GRE, although the most tapered, showed a very large diameter at any
419 depth (Figs. 3a and 4). NOINT also developed large diameters along the taproot. In these three straight
420 populations, the taproot was remarkably developed and able to play a major role in anchorage. Further
421 investigations are required to determine whether adaptations to mechanical stresses such as wind or the
422 ability to colonize deeper soils in the region of origin are related.

423 Despite the high variability in total biomass shown by the straight populations, straight
424 populations had significantly heavier stems and lighter branches than twisted populations. In particular,
425 the LEIR and GRE populations are distinguished by the greatest proportion of SDW and lowest
426 proportion of BDW. These different resource distributions may be related to the greater straightening
427 efficiency of the stems in the straight-stemmed populations driven by secondary growth and the formation
428 of compression wood (Sierra de Grado et al. 2008). Compression wood is denser than normal wood (Gryc
429 and Horáček, 2007), and differences in the density of CW among populations and individuals might exist.

430 A lower proportion of branch biomass may be beneficial to the plant's ability to resist wind. It is
431 remarkable that the difference between straight and twisted populations results from the stem/branch
432 biomass ratio and that the aboveground/underground ratio, by contrast, was very uniform across all
433 populations.

434 Despite the high score of the plants from BUSO as a straight population in provenance tests
435 (Alia et al. 1995), it appears that their small size is the only remarkable trait among the studied characters.
436 A very sensitive response (reducing or stopping growth when encountering mechanical stimulus), or
437 some other feature not included in this experiment, might be an additional strategy involved in
438 straightness. The ALMO population was considered a straight population based on the provenance test
439 data (Alia et al. 1995), but in our experiment, its performance was very close to that of the twisted
440 populations, as shown in the PCA and dendrogram. However, ALMO (followed by NOINT) is the
441 provenance with highest proportion of local thickenings, which is a way of strengthening the second-
442 order roots.

443 The great plasticity of roots has led several authors to consider them as opportunistic entities, the
444 development of which depends on heritable characteristics of species, soil properties (much more
445 heterogeneous as an environment than air) and other environmental factors (Atger 1994; Bowen 1985;
446 Pavlis and Jenik 2000; Jourdan et al. 2000). The existence of a genetic control for the construction of the
447 roots and anchorage into the soil may justify the “inherently regular pattern structure of the roots”
448 discussed by Henderson et al. (1983) Khuder et al. (2007) and Danjon et al. (2013) and provides the basis
449 for the development of genetic differences that may lead to the existence of individuals and populations
450 with varying strategies to address mechanical stresses. Our results appear to reinforce this understanding,
451 with straight-stemmed populations showing different ways of response and a potentially increased
452 responsiveness. In particular, we observed three possible additional distinguishing characteristics of
453 various straight-stemmed populations: a) strengthening of the taproot by developing a thick cylindrical
454 taproot and tapered lateral roots (NOINT, LEIR); b) strengthening of the second-order roots by
455 developing locally thickened segments in the areas of the roots under tension (ALMO, NOINT); and c)
456 allocating biomass preferentially in the stem instead of in the branches (GRE, LEIR).

457

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Table 1. Studied populations and their codes: 01 to 05, twisted-stemmed populations; 06 to 10, straight-stemmed populations. All of the populations are in Spain except for Leiría (Portugal).

Population code	Provenance region	Typical stem form
01ONA	Sierra de Oña	Twisted
02NIEV	Meseta Castellana	
03ESPA	Sierra de Espadán	
04ALMI	Sierra Almirajara - Nevada	
05SEGU	Sierra de Segura - Alcaraz	
06ALMO	Serranía de Cuenca	Straight
07NOINT	Noroeste Interior	
08BUSO	Montaña de Burgos-Soria	
09GRE	Sierra de Gredos	
10LEIR	Leiría	

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Table 2. Acronyms and short descriptions of the studied traits. Units are indicated in brackets; otherwise, the variable is dimensionless.

Variable acronym	Description
DTR0, DTR10, DTR25	Diameter of the taproot at 0, 10 and 25 cm deep [mm]
EC0, EC10, EC25	Cross-sectional eccentricity of the taproot at 0, 10 and 25 cm deep
DSR0, DSR10**	Diameter of second-order roots at the insertion point (0) and 10 cm away [mm]
pDTR10, pDTR25, pDSR0, PDSR10	Diameters of the taproot and second-order roots as a percentage of DTR0 (%)
ppDSR10	DSR10 as a percentage of DSR0 (%)
TTR, TSR**	Taper of taproot, taper of second-order roots
THICK	Presence of thickening in each second-order root
NSR	Number of coarse second-order roots
SDW	Stem dry weight [gr]
BDW	Branches and foliage dry weight [gr]
CRDW	Coarse roots dry weight [gr]
FRDW	Fine roots dry weight [gr]
AGDW/BGDW	Above-ground dry weight/Below-ground dry weight
BDW2, SDW2, CRDW2, FRDW2	Biomass fractions as a percentage of the total plant biomass

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Table 3. P-values for provenance factor and orthogonal contrasts for variables measured on taproots and orthogonal contrasts between straight-stemmed and twisted-stemmed provenances.

	Provenance (p-value)	Contrasts between straight-stemmed and twisted-stemmed provenances
DTR0	0.0008	0.4096
DTR10		0.1571
DTR25	0.0260	0.0802
TTR	0.0251	0.2154
EC0	0.3540	0.6580
EC10	0.3029	0.7330
EC25	0.3528	0.4711
pDTR10	0.0076	0.3317
pDTR25	0.6696	0.1582

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Table 4. P-values of mixed models for variables measured on second-order roots and orthogonal contrasts between straight-stemmed and twisted-stemmed provenances.

	Provenance (p-value)	Sector (p-value)	Provenance x sector (p-value)	Contrast Straight- vs. Twisted- stemmed provenances
DSR0	<0.0001	0.0263	0.9609	0.2513
DSR10	0.0471	0.0485	0.9103	0.6136
TSR	0.0083	0.0002	0.2972	0.0136
pDSR0	0.4061	0.1304	0.9968	0.3767
pDSR10	0.0243	0.0322	0.9697	0.6235
ppDSR10	0.0258	0.0028	0.6546	0.0333

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Table 5. Results of the log linear model for the number of coarse second-order roots (NSR) by provenance, sector and presence of thickened segments.

Maximum likelihood analysis of variance			
Source	DF	Chi-Square	Pr > ChiSq
Provenance	9	13.86	0.1274
THICK	1	533.83	<0.0001
Provenance x THICK	9	19.51	0.0212
Sector	3	19.53	0.0002
Provenance x Sector	27	22.02	0.7364
Sector x THICK	3	23.63	<0.0001
Likelihood ratio	27	37.57	0.0849

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613 Table 6. Orthogonal contrast between straight-stemmed and twisted-stemmed provenances for biomass
614 fractions.

Straight - twisted	Estimator	Standard Error	Pr > t
TOTAL	0.8060	8.3188	0.9229
Above-ground	0.7184	6.5221	0.9124
Branches	-7.9876	4.0342	0.0485
Stem	8.7060	3.2520	0.0078
Below-ground	0.0877	2.2279	0.9685
Coarse roots	1.5280	1.1572	0.1875
Fine roots	-1.4403	1.3317	0.2802

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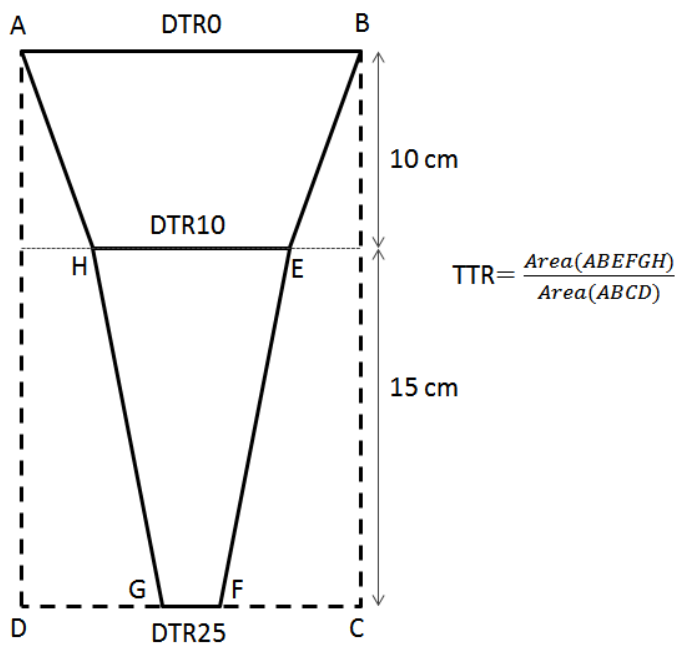
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Table 7. Coefficients of correlation between variables (calculated for plant individual values, n=100) and level of significance (p-value below). Variables mDSR0 and mDSR10 are the mean values of all measured second-order roots per plant. PTHICK is the ratio between the number of thickened segments and the number of second-order roots per plant.

	DTR0	DTR10	DTR25	TTR	DSR0	DSR10	TSR	BDW	SDW	CRDW	FRDW	BDW2	SDW2	CRDW ₂	FRDW2	NRS
DTR10	0.6835															
	0.0000															
DTR25	0.5348	0.7086														
	0.0000	0.0000														
TTR	-0.1112	0.6080	0.5684													
	ns	0.0000	0.0000													
DSR0	0.5662	0.3526	0.2578	-0.1002												
	0.0000	0.0003	0.0096	ns												
DSR10	0.4780	0.1863	0.1719	-0.2212	0.7791											
	0.0000	ns	ns	0.0270	0.0000											
TSR	-0.0186	-0.2015	-0.1134	-0.2449	-0.1806	0.4274										
	ns	0.0444	ns	0.0140	ns	0.0000										
BDW	0.7515	0.5058	0.3901	-0.0834	0.4451	0.4792	0.1408									
	0.0000	0.0000	0.0001	ns	0.0000	0.0000	ns									
SDW	0.6453	0.4271	0.2704	-0.0754	0.5397	0.3951	-0.1131	0.4471								
	0.0000	0.0000	0.0065	ns	0.0000	0.0000	ns	0.0000								
CRDW	0.7540	0.6237	0.5125	0.0666	0.5275	0.4254	-0.0229	0.5853	0.6710							
	0.0000	0.0000	0.0000	ns	0.0000	0.0000	ns	0.0000	0.0000							
FRDW	0.6454	0.4427	0.5339	-0.0010	0.3757	0.4281	0.1983	0.6518	0.3465	0.5179						
	0.0000	0.0000	0.0000	ns	0.0001	0.0000	0.0479	0.0000	0.0004	0.0000						
BDW2	0.1578	0.0800	0.0259	-0.0793	-0.0690	0.0536	0.1687	0.5998	-0.3346	-0.1224	0.1589					
	ns	ns	ns	ns	ns	ns	ns	0.0000	0.0007	ns	ns					
SDW2	0.1312	0.0891	-0.0244	-0.0246	0.2740	0.1297	-0.1952	-0.1904	0.7130	0.1744	-0.1926	-0.7055				
	ns	ns	ns	ns	0.0058	ns	ns	ns	0.0000	ns	ns	0.0000				
CRDW2	-0.1160	0.0303	0.0396	0.1378	-0.0477	-0.0981	-0.0630	-0.3216	-0.1135	0.3951	-0.2529	-0.4068	-0.0406			
	ns	ns	ns	ns	ns	ns	ns	0.0011	ns	0.0000	0.0111	0.0000	ns			
FRDW2	-0.3663	-0.2933	-0.0335	0.0539	-0.2922	-0.2138	0.0974	-0.3828	-0.5242	-0.4036	0.2612	-0.1199	-0.4579	-0.0957		
	0.0002	0.0031	ns	ns	0.0032	0.0326	ns	0.0001	0.0000	0.0000	0.0087	ns	0.0000	ns		
NRS	0.2376	0.2233	0.0562	-0.0134	0.0061	0.0407	0.0666	0.2340	0.1409	0.3115	0.2730	0.0448	-0.0341	0.1017	-0.0976	
	0.0173	0.0255	ns	ns	ns	ns	ns	0.0191	0.1620	0.0016	0.0060	ns	ns	ns	ns	
THICK	0.0554	0.1347	-0.0582	0.0613	0.1383	0.1260	0.0750	0.0221	0.2006	0.2308	-0.0372	-0.1139	0.1353	0.1442	-0.1530	0.0197
	ns	ns	ns	ns	ns	ns	ns	ns	0.0453	0.0209	ns	ns	ns	ns	ns	ns

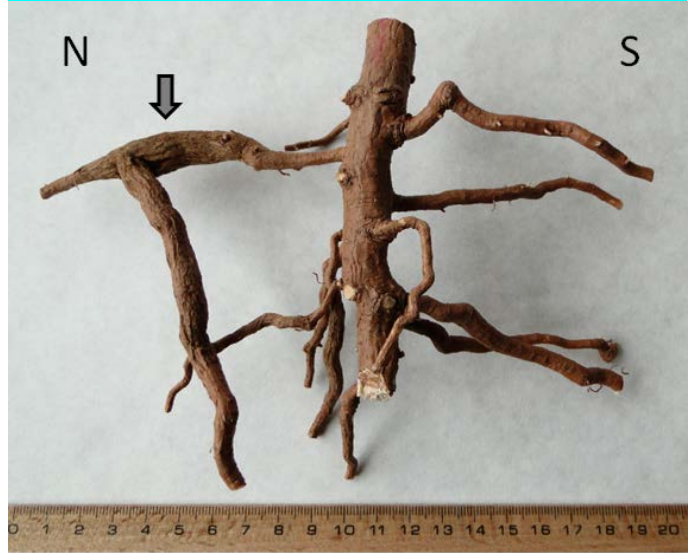
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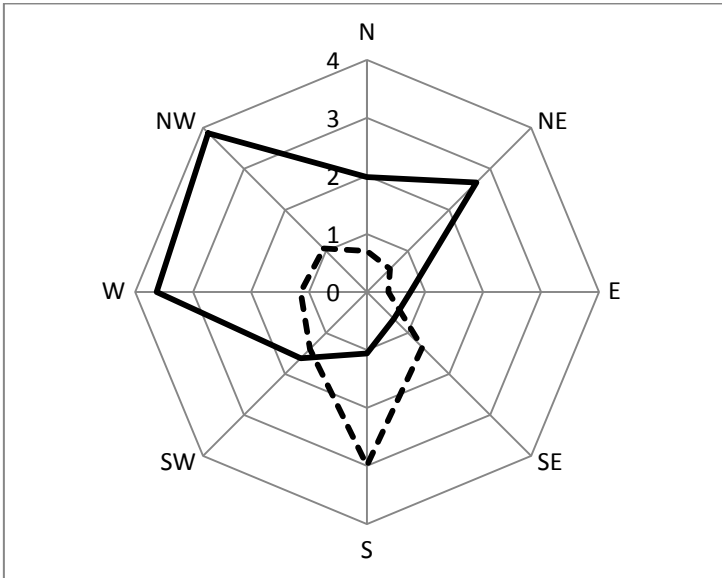


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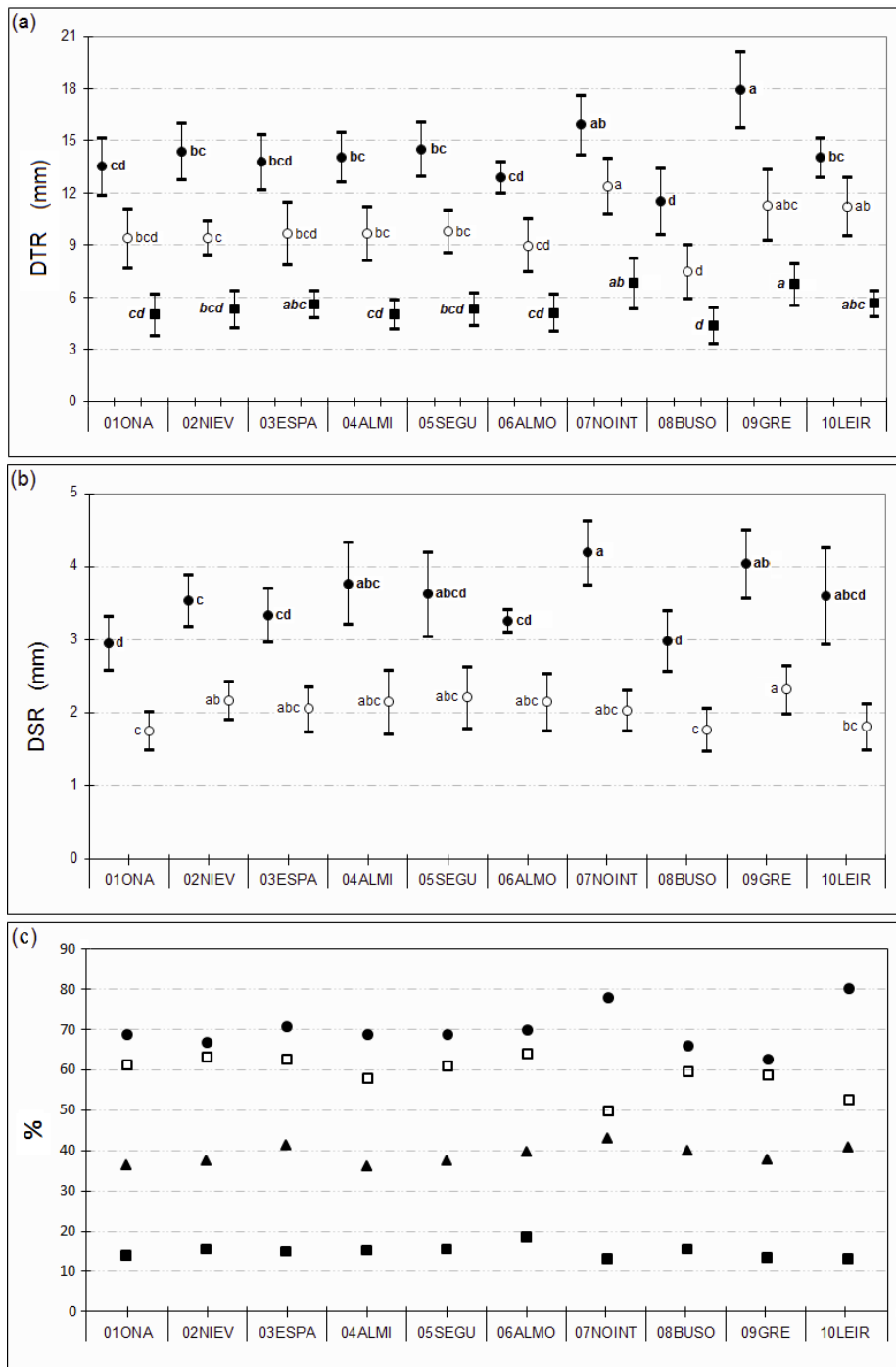
Figure 1. Illustration of the computation of the taper index of the taproot (TTR)



630 Figure 2. (a) Root system in inverted position in the device for measuring.
 631 (b) Thickening developed on some second-order root segments.
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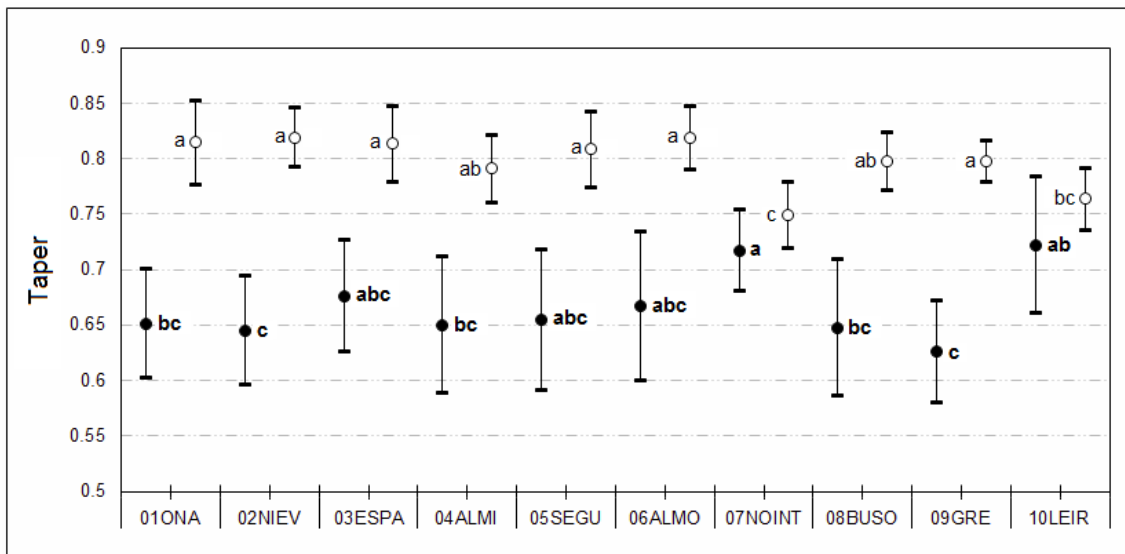
633 Figure 3. Compass rose in the experimental site during the course of the experiment.
 634 Solid line: Winds during the warmer months (from June to September 2009). Dotted
 635 line: Winds during the colder months (from October 2009 to February 2010 included).
 636 Units in the axis indicate the absolute number of records in each direction in thousands.
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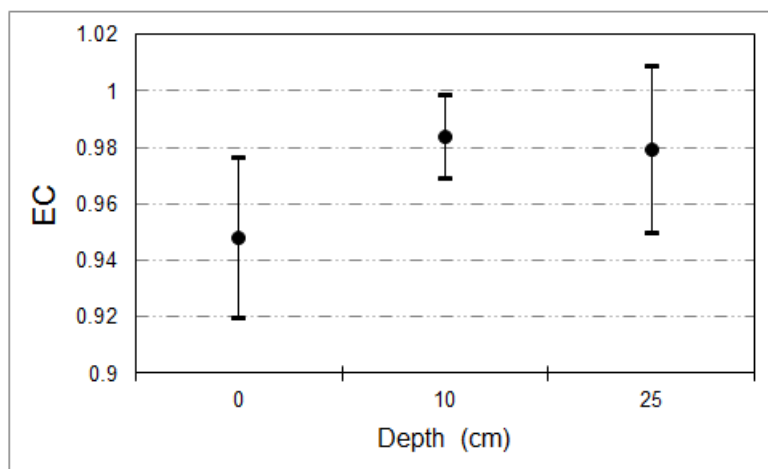
Figure 4. (a) Mean plot for diameters of the taproot at depths of 0, 10 and 25 cm. Filled circles: DTR0; empty circles: DTR10; filled squares: DTR25. (b) Diameters of second-order roots at the insertion point and 10 cm away. Filled circles: DSR0; empty circles: DSR10. (c) Diameters of the taproot and second-order roots as a percentage of DTR0 and DSR10 as a percentage of DSR0. Error bars represent 95% confidence intervals. Within the same variable, means followed by the same letter do not differ significantly at the 5% level among provenances.

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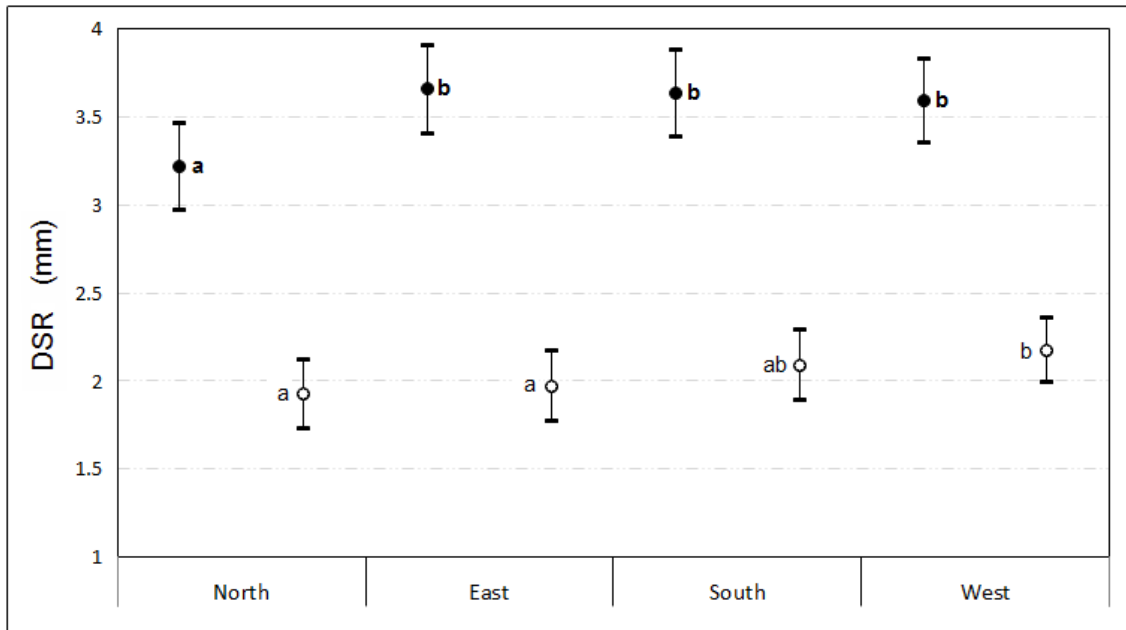
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Figure 5. Taper of the taproot and second-order roots by provenance. Filled circles: TTR; empty circles: TSR. Error bars represent 95% confidence intervals. Within the same variable, means followed by the same letter do not differ significantly at the 5% level among provenances.



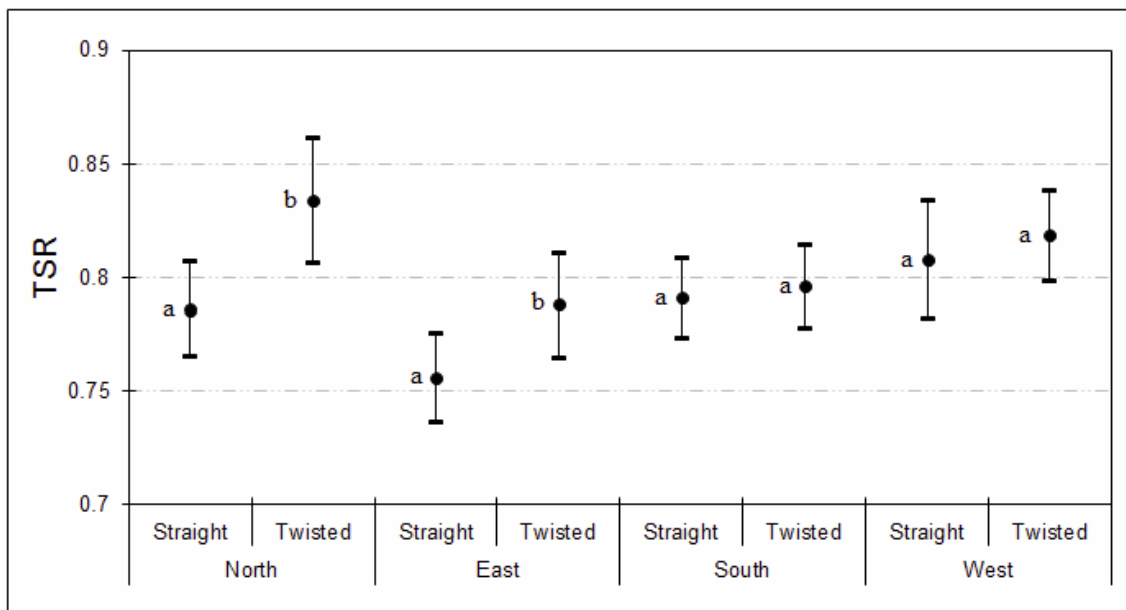
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Figure 6. Mean plots of the eccentricity of the taproot (ratio between WE and NS diameters) at 0, 10 and 25 cm depth (EC0, EC10 and EC25, respectively). Error bars represent 95% confidence intervals.



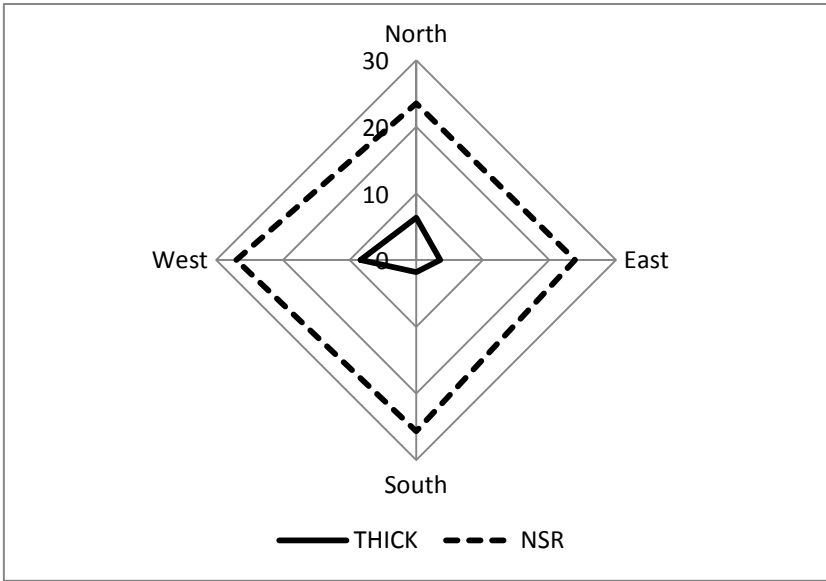
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Figure 7. Mean plots of diameters of second-order: DSR0 (filled circles) and DR10 (empty circles), by sector. Error bars represent 95% confidence intervals. Within the same variable, means followed by the same letter do not differ significantly at the 5% level among sectors.



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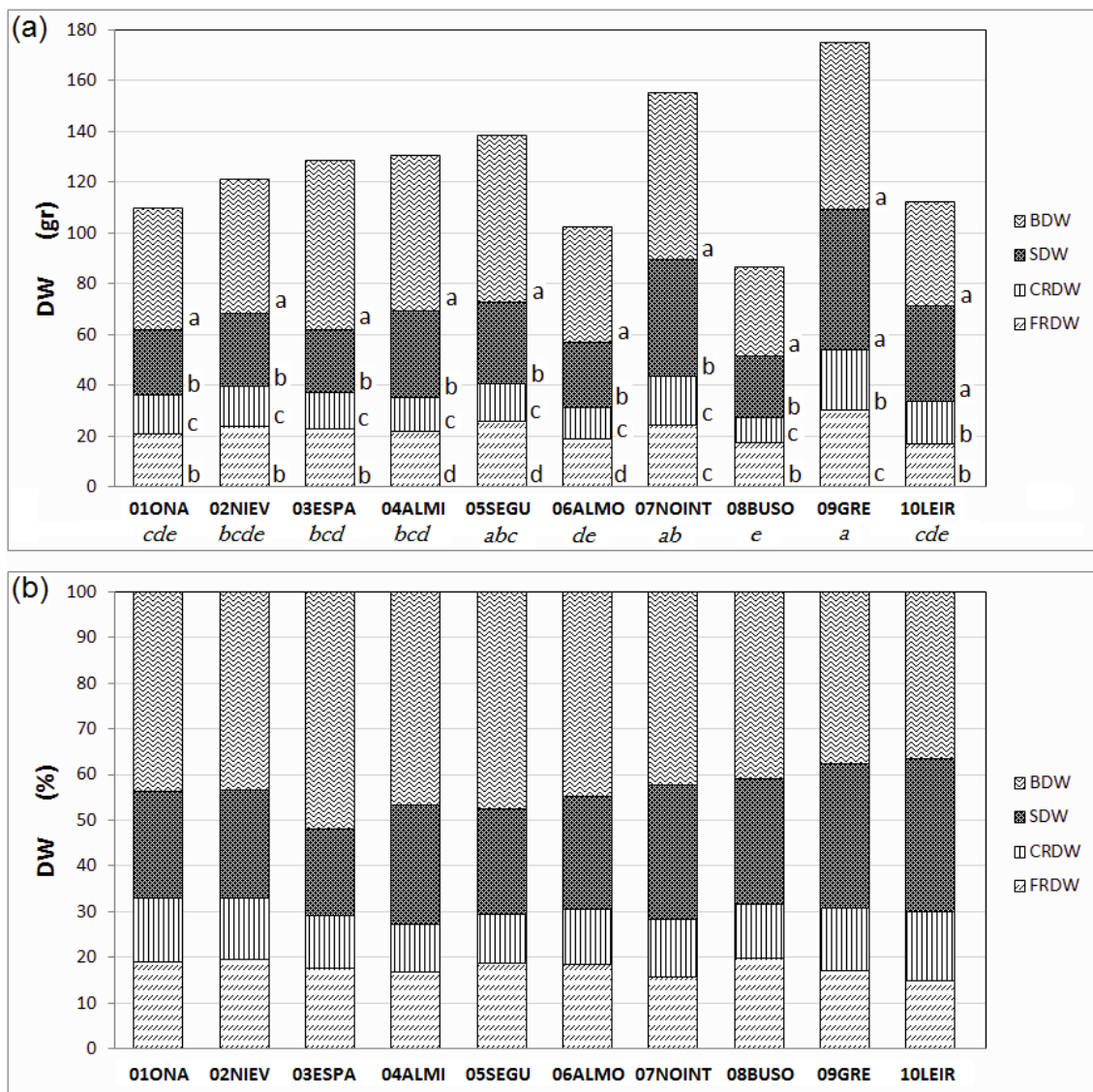
Figure 8. Mean plots of the taper of second-order roots (TSR) by sector in straight and twisted-stemmed provenances. Error bars represent 95% confidence intervals. Within the same variable, means followed by the same letter do not differ significantly at the 5% level between straight and twisted populations in the same sector.



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Figure 9. Percentage of second-order roots (NSR) per sector and percentage of second-order roots with thickenings (THICK) per sector.

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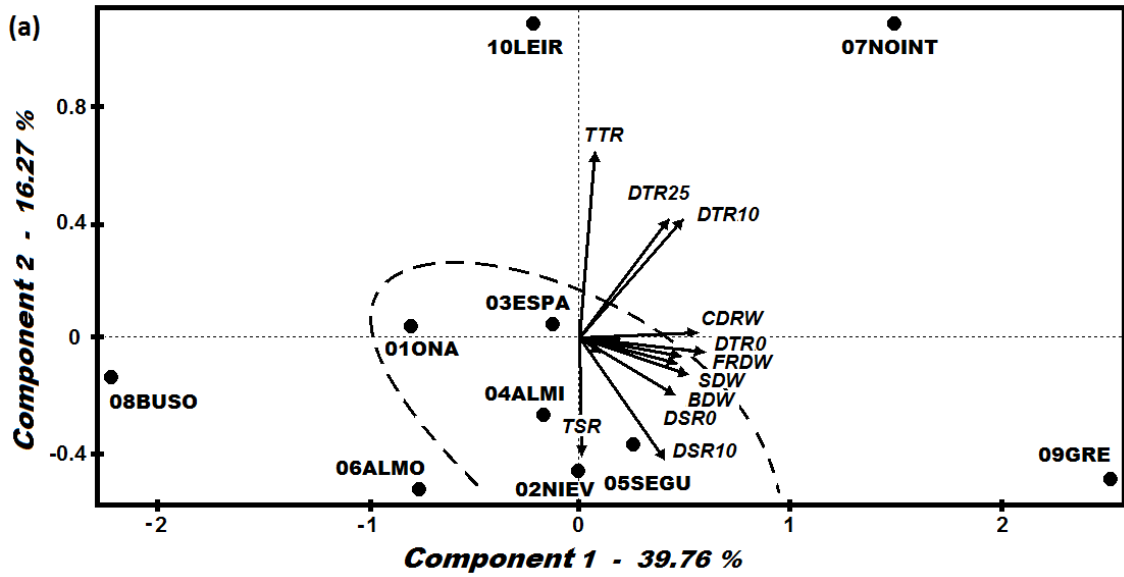


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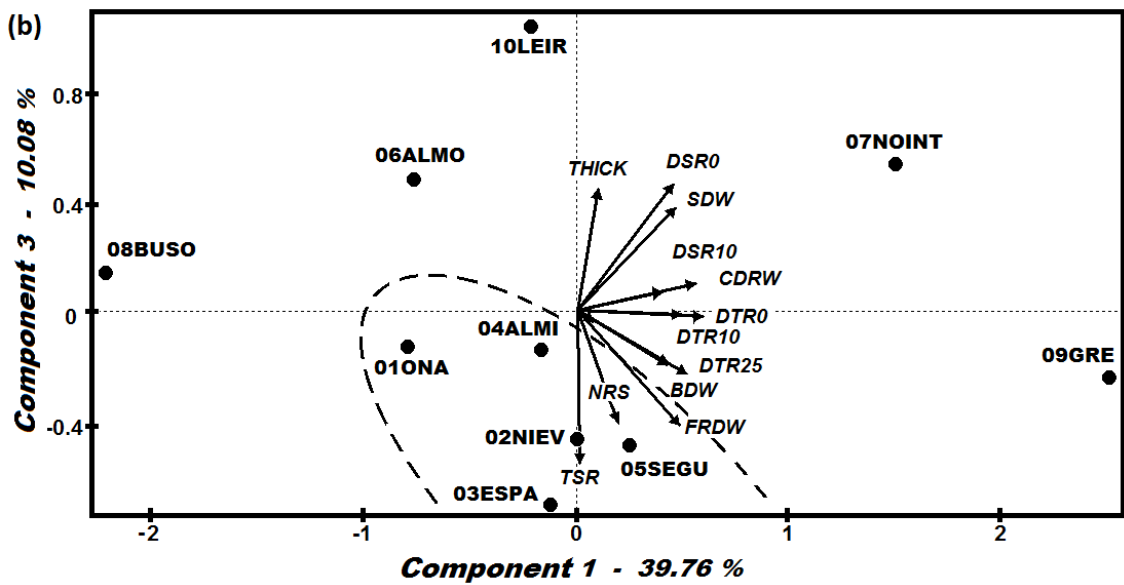
Figure 10. Biomass partitioning among four fractions [branches (BDW), stem (SDW), coarse roots (CRDW) and fine roots (FRDW)] by provenance (a): absolute values; (b): relative values respect to the total biomass. Coarse roots include taproot and second-order roots thicker than 2 mm in diameter, between 0 and 25 cm deep and in a radius of 10 cm around the main axis of the taproot; the fine roots are roots not included in the previous class. Means followed by the same letter do not differ significantly at the 5% level; letters beside bars indicate differences between fractions within the same provenance; and letters under the names of the provenances indicate differences in total biomass between provenances.

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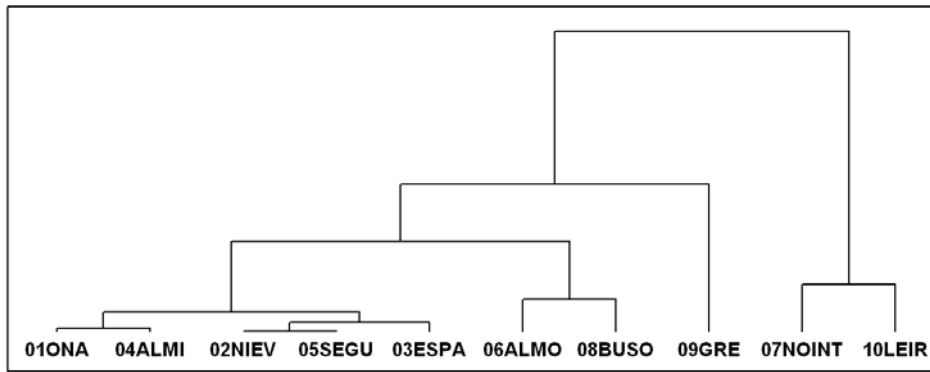
Figure 11. Plots of variables and populations in the PCA planes (a: F1x2; b: F1x3; F1, F2 and F3 are the first three components). Dotted line includes twisted-stemmed populations.



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Figure 12. Dendrogram of the populations from a principal component analysis with the variables that showed significant differences among provenances. Three factors were retained, explaining 66.11% of the observed variation.