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Adventitious root formation in *Castanea* sp. semi-hard cuttings is under moderate genetic control caused mainly by non-additive genetic variance

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1 Adventitious root formation in *Castanea* sp. semi-hard cuttings is under
2 moderate genetic control caused mainly by non-additive genetic variance

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Draft

7 Abstract

8 Breeding programmes of European chestnut for disease resistance have focused on
9 interspecific hybridization followed by clonal propagation. Although chestnut species are
10 recalcitrant to rooting by cuttings, it is possible to mass propagate new softwood cuttings taken
11 from shoot stumps. To determine the importance of genetic control in adventitious root formation in
12 chestnut, two trials were conducted with 705 ortets from 25 young full-sib families
13 generated by combining 11 different parents including seven *C. sativa*, one *C. crenata*, one *C.*
14 *mollissima* and two F1 hybrid (*C. crenata* × *C. sativa*) individuals. Rooting variables were
15 analysed using an incomplete diallel model and statistical analyses were performed using the
16 MIXED and GLIMMIX procedures for continuous and binomial variables, respectively.

17 Very high rooting percentages were obtained (91% and 82%) and the cuttings showed
18 adequate numbers of roots (17 and 10); both characteristics were correlated ($\hat{r}_G = 0.93$)
19 and presented moderate genetic control ($\hat{H}^2 = 0.33$ for Proot; 0.43 and 0.35 for Nroots)
20 due mainly to dominance and epistasis ($\hat{h}^2/\hat{H}^2 = 0.21$ for Proot; 0.23 and 0.27 for
21 Nroot). There were good rooting ortets within all families and, accordingly, it is possible
22 to select for rooting capability within each family, at least at a young age.

23 **Keywords:** adventitious roots, *Castanea* sp., cuttings, rooting ability, non-additive variance

24 Introduction

25 Breeding programmes involving controlled pollination of individuals replicated by clonal methods
26 provide an opportunity to estimate the genetic control of target quantitative traits and its additive
27 and non-additive components, and, furthermore, to quantify the importance of dominance and
28 epistasis, the two components of non-additive genetic variance. Additive genetic variance,
29 responsible for similarity between relatives, determines the heritability of traits and is caused by the
30 sum of the effects of different genes that combine their effects, while dominance is due to
31 interactions between alleles of a locus and epistasis is due to interaction between alleles of different
32 loci (Lynch and Walsh 1998). The rooting ability of cuttings, a polygenic or quantitative trait
33 (White et al., 2007), is very important in breeding programmes for trees that use vegetative
34 propagation to obtain maximum gains by better utilizing additive genetic variance and complete
35 capture of non-additive genetic variance of target selection traits (White et al., 2007).

36 European chestnut (*Castanea sativa* Mill.) is a rooting-recalcitrant tree when propagated by
37 cuttings. It is a dual purpose species because it produces nuts and high quality wood. Occurs
38 naturally and is cultivated in southern Europe around the Mediterranean Sea. Because of its
39 sensitivity to ink and canker diseases (*Phytophthora* spp. and *Chryphonectria parasitica*,
40 respectively), several breeding programmes have sought to incorporate resistance into European
41 chestnut through hybridization with Chinese and Japanese chestnut (*Castanea mollissima* and
42 *Castanea crenata*, respectively) (Salesses et al. 1993; Fernández-López 2011; Costa et al. 2011;
43 Santos et al. 2015; Míguez-Soto et al. 2016). Clonal propagation methods are used to multiply
44 chestnut individuals obtained by breeding with the aim of fixing certain productive traits and
45 tolerance to diseases and pests in varieties to be mass propagated in clonal forestry or as rootstocks
46 or nut producers. Therefore, materials selected for other characteristics also need to have good
47 vegetative propagation ability.

48 Clonal propagation of chestnut trees to obtain new plants is carried out by layering (Schad et al.,
49 1952), *in vitro* culture (Viéitez et al. 2007; Miranda-Fontaiña and Fernández-López, 2001; Saez et
50 al. 2013; Giovanelli and Giannini 2000;) or cuttings. Cutting propagation has been studied for
51 decades as a method of asexual multiplication for different chestnut species (Hunter and Norton
52 1985) and inter-specific hybrids (Viéitez et al., 1999). The Breeding Programme of Chestnut trees
53 in Galicia (NW Spain) has been using cuttings to multiply different genotypes since 1991
54 (Fernández-López et al. 1992) because of the simplicity, low cost and short time required for the
55 propagation of many different materials, which is often required in breeding programmes.

56 Adult chestnut trees have been classified as recalcitrant to propagate by cuttings (Urquijo 1952;
57 Graves and Nienstaedt. 1953; Viéitez 1999) because of several factors related with the decreased
58 ability to form adventitious roots, including the presence of a continuous sclerenchyma ring in
59 lignified cuttings (Biricolti et al. 1994; Ballester et al. 1999), the presence of methylated derivatives
60 of ellagic acid (Viéitez et al. 1987), and finally starch reduction within cuttings (Fernández-Lorenzo
61 et al. 2005).

62 Thus, cuttings are propagated in spring-summer with juvenile unlignified material, using new
63 shoots formed during the current growing season from normal or adventitious buds of the plant or
64 the trunk base of young or adult individuals. The endogenous auxin in the cuttings is insufficient to
65 induce root formation, but this can be mitigated by applying indole-3-butyric acid (IBA), which is
66 resistant to degradation by enzymes present in the internal tissues of the cuttings and thus remains
67 inside them longer (Nordström et al., 1991). During the rooting process until the emergence of the
68 roots, softwood cuttings can die from desiccation if the environmental humidity within the
69 propagation chamber is too low. To avoid this, a misting *fog-air* system can be used to maintain a
70 moist atmosphere, thereby decreasing water loss through transpiration (Ponchia and Howard 1987;
71 Fernández-López et al. 1992; Štefančič et al. 2007). Under these conditions, 60-year-old clones

approved as forest reproduction materials (Fernández-López, 2011) have a mean rooting success of 63%, with 80% for the best rooting clones; values far from desirable for mass clonal propagation.

Current research interest in the rooting ability of chestnut trees is focused on known candidate genes involved in rooting and how they act, with a view to improving this characteristic through biotechnology (Rinallo et al. 1993; Sánchez et al. 2007; Vielba et al. 2011). This indicates that the rooting ability of chestnut trees is considered a limiting factor in breeding or development programmes.

Although chestnut cuttings have been used for decades, there have been few quantitative genetic studies related to the genetic control of rooting ability, which would allow us to estimate the difficulties that poor rooting may cause in genetic improvement programmes and determine how to handle them. Studies on the genetic control of rooting ability by micropropagation have focused on specific chestnut hybrid clones without considering family structure; the broad-sense heritability values obtained indicate high genetic control of rooting and the possibility of achieving large genetic gains by selecting the best rooting clones (Miranda-Fontañá and Fernández-López 2005).

The development of a new breeding programme involving controlled pollination of a number of parents selected for other characteristics, such as resistance to *Phytophthora* spp. and wood or nut quality (Míguez-Soto et al., 2016), offers the opportunity to study how the characteristics of rooting ability and the quality of the root system are distributed in the breeding population and obtain information on their genetic control.

We carried out an experiment with young progenies for two consecutive years to: (1) determine the importance of genetic control in rooting ability and root system quality, and its additive, dominance and epistatic components, as well as to estimate the breeding values of the parents, families and individuals within each family for rooting traits, and (2) estimate the correlations between different rooting variables.

96 Materials and Methods

97 *Plant material*

98 The plants used in the rooting trials were obtained from seeds generated by controlled pollinations
99 in 2010 and 2011. The design used for the controlled pollinations was an incomplete diallel in
100 which the female and male parents were considered equivalent, so that reciprocal crosses and self-
101 crosses were omitted. The parents used for these pollinations came from three breeding groups of
102 *Castanea* spp. belonging to the Elite Population developed at the Forestry Research Centre of
103 Lourizán. These groups were the wood quality group, the resistance to *P. cinnamomi* group and the
104 vigour group.

105 The controlled pollinations in 2010 (Míguez-Soto et al. 2016) resulted in 16 families (Table 1A).
106 The female parents were six individuals; four traditional *C. sativa* varieties (Paredel, Paredelb,
107 Presa and Garrida1a) (Fernández-López and Fernández-Cruz 2015) and two F1 hybrids of *C.*
108 *crenata* × *C. sativa* (CA-15 and 2522), chosen for their high resistance to *P. cinnamomi* (Miranda-
109 Fontaíña et al. 2007) and their vigour and straightness. Five male parents were used; one traditional
110 *C. sativa* variety (Presa), two F1 hybrids of *C. crenata* × *C. sativa* (CA-15 and 2522), one *C.*
111 *crenata* (23M09) chosen for its high resistance to *P. cinnamomi* and one *C. mollissima* (M5M) also
112 chosen for its resistance to *P. cinnamomi*. The controlled pollinations in 2011 resulted in nine
113 families (Table 1B). Three female parents were used: one *C. sativa* (Paredel) and two F1 hybrids of
114 *C. crenata* × *C. sativa* (CA-15 and 2522). Three male parents were also used: three *C. sativa*
115 individuals (21-LU-29, 5-LU-29 and 19-C-17) from the progenies of plus trees selected on the
116 Galician coast chosen for their vigour and form (Míguez-Soto and Fernández-López 2012). The 25
117 families used in the rooting experiments were grouped into the following genealogical classes: 12
118 families were backcrosses of an F1 hybrid, *C. crenata* × *C. sativa*, to four *C. sativa* individuals,
119 onwards BC(CcxCs)Cs; two families were backcrosses from an F1 hybrid, *C. crenata* × *C. sativa*,
120 to a *C. crenata* individual, BC(Cc × Cs)Cc; three families were F1 hybrids, *C. crenata* × *C. sativa*,

F1(Cs x Cc); one family was an F1 hybrid, *C. sativa* × *C. mollissima*, F1(Cs x Cm); one family was an F2 hybrid, *C. crenata* × *C. sativa*, F2(Cc x Cs); two families were crosses between an F1 hybrid, *C. crenata* × *C. sativa*, with a *C. mollissima* individual, (Cc x Cs)Cm; and four families were crosses between *C. sativa* individuals, Cs x Cs (Table 1).

The initial plants used as stock mother plants for cuttings were obtained from the seeds of controlled pollinations. These plants were coppiced annually during late winter to induce more outbreaks of adventitious buds from the base. These new shoots, developed from a mix of normal and adventitious buds, were used to obtain cuttings for the rooting trials (Fig. 1).

To study the genetic control of rooting ability, two trials were performed in the spring-summer of 2012 (Assay1) and 2013 (Assay2). The cuttings used in Assay1 were collected from one-year-old plants of 16 families originating from the controlled pollinations made in 2010. In this assay, the average number of individuals per family was 18, with a minimum of seven and a maximum of 27, and a total of 294 individuals (Table 1). The cuttings for Assay2 were collected from two-year-old plants of 16 families made in 2010 and nine families of one-year-old plants made in 2011. Assay2 included an average number of individuals per family of 16, with a minimum of six and a maximum of 23, and 258 individuals from 2010 families and 153 individuals from 2011, for a total of 411 (Table 1).

In both trials, six cuttings of each individual were used, yielding a total of 1770 and 2466 cuttings in assays 1 and 2, respectively. Placing the Assay1 took 15 days, from 21 May to 3 June 2012. Data collection was carried out from 31 July to 31 August of the same year, and the cuttings remained in the propagation greenhouse tunnels an average of 80 days. Placing the Assay2 lasted three weeks, from 13 May to 3 June 2013. The data collection was carried out from 16 July to 8 August of the same year; in the second trial, the cuttings remained in the tunnels an average of 65 days. In the Assay1, 96 cuttings of clone 2522 (F1 hybrid of *C. crenata* x *C. sativa*) were also used like rooting

control. In the Assay 2, 165 cuttings were used from each of the four clones F1 hybrids of *C. crenata* x *C. sativa* (103, X, 420 and 7521) as controls.

Cutting methods

For the rooting of the cuttings we used black polyethylene trays (60 cm long × 25 cm wide × 15 cm deep). The trays were filled with perlite, an inert substrate with easy drainage. Later, the trays were placed in 8 m long and 1.2 m wide rooting tunnels. The facilities and their management are described in Miranda-Fontañá and Fernández-Lopez (1992). Six rooting tunnels were situated inside a glasshouse in which the temperature was kept at about 24° C using a heating system, a programmable thermal screen under cover and an evaporative cooling system. Inside the tunnels, the moisture was controlled by a *fog-air* system that maintained the humidity at 90–100% saturation during daylight hours; at both ends of the tunnels there were two small 5 W extractors, which started up at the same time as the fog system to evenly distribute the moisture inside each tunnel.

To set the cutting conditions for both experiments, a few small tests were performed in 2012 in which the effects of different concentrations of IBA, different substrates and the position of the cutting in the source shoot on the rooting of juvenile cuttings with leaves were evaluated (López-Villamor et al. 2013).

Cuttings were made with four buds and their four leaves, of which two were eliminated, leaving those in the buds at the top of the cutting. Then, the cuttings were disinfected by dipping the bottom for 2 min in an aqueous solution with fungicide (active ingredient Tiram) at a concentration of 2.5 g/L. Subsequently, the bases of the cuttings were submerged again for 3–5 min in an aqueous solution of IBA as a potassium salt at a concentration of 2 g/L. The water used for both solutions was deionized water. About 30 cuttings were inserted into each polyethylene tray.

At 2 weeks from the start of the assay, fertilization of the trays was initiated to improve the growth and development of cuttings. In the third week, the process of hardening the cuttings by gradually

decreasing the moisture inside the tunnels was started; another goal was to prevent rotting and activate the stomatal function of the leaves. The fertilization was performed with half-strength Murashige and Skoog (1962) solution (Miranda-Fontañña and Fernández-López 1992). The application was performed once a week and fungicide was added every 15 days to prevent rotting. Finally, at 5 weeks from the start of the assay, we collected the data for the rooting variables.

Once the rooting process was completed, the cuttings were removed one by one and the roots were cleaned. The variables recorded included those related to rooting, such as survival, the binary variable presence of root (Proot) and number of roots (Nroots), evaluated in Assays 1 and 2. The roots of the cuttings were then scanned with an A3 image scanner (Epson Expression 10000XL) and the images were analysed using the software package for Windows WinRHIZO™ 2005 a,b (Régent Instruments Inc.) to obtain variables related to root system quality, such as total root length (Trl, measured in cm), average root diameter (Ard, measured in mm) and root volume (Rv measured in cm³), evaluated in Assay2.

Experimental design and statistical analysis

The experimental design was a complete randomized block (RBC) with six replicates (White et al. 2007). In each replicate, all families and individuals were represented by a cutting from each individual, placed randomly. The assay occupied three rooting tunnels with two replicates placed inside each.

In the rooting assays, there were time differences between replicates in the period from the insertion of the cuttings into the rooting substrate to the data collection. To correct for the effect of these differences, the individual values of each variable were standardized to replication level (White et al. 2007). The interaction ‘replicate per family’ ($R \times FM$) was not significant because of standardization, so it was removed from the model.

The variables for rooting ability and root system quality of Assay1 and Assay2 and common families in the two rooting assays were analysed using the SAS statistical package for Windows (SAS version 9.2) with Model1 and Model2, respectively. The analysis of the incomplete diallel (Míguez-Soto et al. 2016) was a modification of the parental model of Isik (2009), in which the individual effect within a family (Model1) was included. In Model2, the age effect and the interactions of different random effects with the age variable were also included. Statistical analyses were performed using the MIXED procedure for continuous variables and the GLIMMIX procedure for presence of root (Proot) with a binomial distribution and a logic link function for binary traits. Variance components were estimated through restricted maximum likelihood (REML) and restricted maximum pseudo-likelihood (REPL) methods in the MIXED and GLIMMIX procedures, respectively. Both statistical procedures also allow the estimation of the best linear unbiased estimate of the fixed effects and the best linear unbiased prediction (BLUP) of the random effects. The linear models used for analysis were:

$$Y_{ijklm} = \mu + R_i + F_j + M_k + FM_{jk} + I(FM)_{l(jk)} + \varepsilon_{ijklm} \text{ (Model1)}$$

$$Y_{ijklmn} = \mu + R_i + a_m + F_j + M_k + FM_{jk} + I(FM)_{l(jk)} + a \times F_{mj} + a \times M_{mk} + a \times FM_{mjk} \\ + a \times I(FM)_{ml(jk)} + \varepsilon_{ijklmn} \text{ (Model2)}$$

where Y_{ijklm} (Model1) and Y_{ijklmn} (Model2) are the response variables of the m^{th} and n^{th} ramet respectively, from the l^{th} ortet (individual) within full-sib family jk in the replicate i ; μ is the average value of the studied population; R_i is the fixed effect of replicate i ; a_m is the fixed effect of age m ; F_j and M_k are the general combining ability (GCA) of the random effect of the j^{th} female parent and k^{th} male parent; FM_{jk} is the random effect of the female parent j combined with the male parent k (specific combining ability (SCA)); $I(FM)_{l(jk)}$ is the random effect of ortet (individual) l within family jk ; $a \times F_{mj}$ and $a \times M_{mk}$ are the random effects of the interactions of age with the female parent j and male parent k (GCA), respectively; $a \times FM_{mjk}$ and $a \times I(FM)_{ml(jk)}$ are the

random effects of the interactions of age m with family jk (SCA) and the individual m within family jk , respectively; ε_{ijklm} (Model1) and ε_{ijklmn} (Model2) are the experimental errors.

The significance of the analysis of variance was tested using the likelihood ratio test (LRT) (Kendall and Stuart 1979):

$$\text{LRT} = -2 \times (\log L. \text{ of full model} - \log L. \text{ of reduced model}).$$

In the above formula, the complete model contains the effect that we want to evaluate and the reduced model is the incomplete model without this effect. Under the null hypothesis, it is expected that the value of LRT is distributed as χ_q^2 with q degrees of freedom given by the difference in the numbers of parameters between the two models. When $\text{LRT} > \chi_q^2$, the estimation of the variance component is significant. LRT was used because it has better statistical properties than the approximation of the Wald test that is given by default in the output of the MIXED procedure (Self and Liang 1987).

The genetic parameters were estimated using the IML procedure in SAS and the following formulas according to Foster and Shaw (1988):

$$\hat{V}_A = 2(\hat{\sigma}_F^2 + \hat{\sigma}_M^2) = \hat{V}_A + 1/4 \hat{V}_{AA} + 1/16 \hat{V}_{AAA} + \dots$$

$$\hat{V}_D = 4 \hat{\sigma}_{FM}^2 = \hat{V}_D + 1/2 \hat{V}_{AA} + 1/2 \hat{V}_{AD} + 1/4 \hat{V}_{DD} + \dots$$

$$\hat{V}_I = \hat{\sigma}_{I(FM)}^2 - (\hat{\sigma}_F^2 + \hat{\sigma}_M^2) - 3 \hat{\sigma}_{FM}^2 = 1/4 \hat{V}_{AA} + 1/2 \hat{V}_{AD} + 3/4 \hat{V}_{DD} + \dots$$

$$\hat{V}_G = (\hat{\sigma}_F^2 + \hat{\sigma}_M^2 + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2)$$

$$\hat{V}_P = \hat{V}_G + \hat{V}_E = \hat{\sigma}_F^2 + \hat{\sigma}_M^2 + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2 + \hat{\sigma}_e^2$$

228 where $\hat{V}_A$, $\hat{V}_D$, $\hat{V}_I$, $\hat{V}_G$ and $\hat{V}_P$ are the additive, dominance, epistatic, genetic and phenotypic
 229 variances, respectively, and $\hat{V}_{AA}$, $\hat{V}_{AD}$ and $\hat{V}_{DD}$ were epistatic genetic variance due to additive x
 230 additive effects, additive x dominance effects and dominance x dominance effects, respectively.
 231 Estimates of additive and dominance genetic variance are biased upward because they are
 232 confounded with fractional components of epistasis and estimates of epistatic genetic variance is
 233 only approximated, and always less than the actual value, because it contains only a fraction of the
 234 total epistasis. For non-continuous variable presence of root (Proot), the residual variance of the
 235 GLIMMIX procedure was set to $\pi^2/3$ (Fahrmeir and Tutz, 1994).

236 The fraction between the genetic dominance variance and the additive genetic variance was
 237 calculated ($\hat{V}_D/\hat{V}_A$) as an indicator of the importance of the additive components or the dominance
 238 components.

239 The narrow-sense heritability ($\hat{h}^2$) and the broad-sense heritability ($\hat{H}^2$) were estimated as:

$$240 \quad \hat{h}^2 = \hat{V}_A/\hat{V}_P = 2 (\hat{\sigma}_F^2 + \hat{\sigma}_M^2) / (\hat{\sigma}_F^2 + \hat{\sigma}_M^2 + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2 + \hat{\sigma}_{FMxB}^2 + \hat{\sigma}_e^2)$$

$$241 \quad \hat{H}^2 = \hat{V}_G/\hat{V}_P = (\hat{\sigma}_F^2 + \hat{\sigma}_M^2) + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2 / (\hat{\sigma}_F^2 + \hat{\sigma}_M^2 + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2 + \hat{\sigma}_{FMxB}^2)$$

242 Standard errors of heritability were calculated using the Delta method (Lynch and Walsh, 1998),
 243 also implemented in IML (Isik 2009). The fraction between narrow-sense heritability and broad-
 244 sense heritability was calculated ($\hat{h}^2/\hat{H}^2$) as an indicator of the suitability of the clonal selection;
 245 “values of $\hat{h}^2/\hat{H}^2$ near one indicate that additive variance is small and advantages of clonal forestry
 246 are reduced compared to the use of seedlings” (White et al. 2007).

247 The phenotypic Pearson correlations ($\hat{r}_p$) between pairs of response variables within each trial were
 248 estimated using the CORR procedure in SAS.

The VARCOMP procedure of SAS (Isik 2009) was used to estimate the genetic correlations (type A). To calculate the $\widehat{cov}_{xy}$ we needed to create a dummy variable that linked the two variables (x and y) through a sum; $z = x + y$. Finally, the correlations ($\hat{r}_A, \hat{r}_D, \hat{r}_I, \hat{r}_G, \hat{r}_e$) were calculated based on their respective covariances, according to Falconer and Mackay (1996):

$$\hat{r}_n = \frac{\widehat{cov}_{n(x,y)}}{\hat{\sigma}_x \hat{\sigma}_y}$$

where n refers to the additive, dominance, epistatic, genetic or environmental component of phenotypic variance.

Thereafter, the standard errors of the type A genetic correlations were calculated with the following formula, according to Falconer and Mackay (1996):

$$SE(r_n) = \frac{1 - r_n^2}{\sqrt{2}} \sqrt{\frac{SE(h_x^2)SE(h_y^2)}{h_x^2 h_y^2}}$$

where $SE(h_x^2)$ and $SE(h_y^2)$ are the standard errors of the individual heritabilities of the variables x and y , respectively.

Type B genetic correlations (Yamada 1962; Burdon 1977), between common variables of the two trials, model 2, were calculated for additive effects, for family and for the total breeding value. The standard errors of these correlations were calculated with the formula of Falconer and Mackay (1996) that was used for genetic correlations (type A). These type B correlations were estimated as follows:

Additive genetic correlation($\hat{r}_A$):

$$\hat{r}_A = \frac{\hat{\sigma}_F^2 + \hat{\sigma}_M^2}{\hat{\sigma}_F^2 + \hat{\sigma}_M^2 + \hat{\sigma}_{a \times F}^2 + \hat{\sigma}_{a \times M}^2}$$

265 Dominance genetic correlation ($\hat{r}_D$):

$$\hat{r}_D = \frac{2(\hat{\sigma}_F^2 + \hat{\sigma}_M^2) + \hat{\sigma}_{FM}^2}{2(\hat{\sigma}_F^2 + \hat{\sigma}_M^2) + 2(\hat{\sigma}_{a \times F}^2 + \hat{\sigma}_{a \times M}^2) + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{a \times FM}^2}$$

266 Clonal correlation or total genetic correlation($\hat{r}_G$):

$$\hat{r}_G = \frac{2(\hat{\sigma}_F^2 + \hat{\sigma}_M^2) + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{I(FM)}^2}{2(\hat{\sigma}_F^2 + \hat{\sigma}_M^2) + 2(\hat{\sigma}_{a \times F}^2 + \hat{\sigma}_{a \times M}^2) + \hat{\sigma}_{FM}^2 + \hat{\sigma}_{a \times FM}^2 + \hat{\sigma}_{I(FM)}^2 + \hat{\sigma}_{a \times I(FM)}^2}$$

267 For a graphical representation of phenotypic and type A genetic correlations, scatter plots of points
268 for the significant effects were created using pairs of variables

269 **Results**

270 ***General observations***

271 The survival rate of the cuttings during the rooting process was very high, both in Assay1 and
272 Assay2, remaining around 99%. The percentage of rooting was also very high for both assays, with
273 values of 91% and 82% for Assays 1 and 2, respectively; in Assay2, 49% of ortets had 100%
274 rooting and 73% of ortets had rooting percentages higher than 85%. Differences were also observed
275 between families: in Assay1, the families Parede1×Presa (Cs×Cs) and 2522×M5M ((Cc×Cs)Cm)
276 had the highest percentages of rooting with 97%, while the family CA-15×23M09 (BC(Cc×Cs)Cc)
277 had the lowest percentage with 72%; in Assay2, the families CA-15×5-LU-29 and CA-15×21-LU-
278 29 (both BC(Cc×Cs)Cs) had the highest percentages of rooting with 93% and 94%, respectively,
279 while the family Presa×23M09 (F1(Cs×Cc)) had the lowest with 48%. In Assay1, the control clone
280 2522 had a rooting percentage of 55% and a survival rate of 96%; in Assay2, all control clones had
281 a survival rate of 100%, while their rooting percentages were: 103 (87%), X (62%), 420 (58%) and
282 7521 (50%), with a mean of 64%.

The average number of roots was 18 and 10 in Assays 1 and 2, respectively (Fig. 2); the control old clone 2522 had a mean of 6 roots. For Assay2, the means of the root system quality variables were 250.56 cm for total root length, 1.18 mm for root diameter and 2.92 cm³ for root volume (Table 2).

Components of the genetic variance, heritability of rooting characteristics and breeding values

Analysis with Model1

In the analysis of variance with Model1, the effects of general combining ability (GCA) and individual within family (I(FM)) for the presence of roots (Proot) were significant in both assays, while the effect of specific combining ability (SCA) was only significant in Assay2 (Table 2). The effects of SCA and I(FM) on the number of roots (Nroots) were significant in both assays, while the effect of GCA was not significant in any case (Table 2). With respect to the root system quality variables, only GCA and I(FM) effects were significant for all variables; total root length (Trl), average root diameter (Ard) and root volume (Rv) (Table 2).

Genetic variance components are also shown in Table 2. The relative importance of additive and dominance components followed the same trends as those shown above between GCA and SCA effects (Fig. 3), because of the proportional formulas used to calculate them. The values of the fraction $\hat{V}_D/\hat{V}_A$ were high for the presence of roots (Proot) (2.43 for Assay2), number of roots (Nroots) (2.6 for Assay1 and 2.83 for Assay2) and root volume (Rv) (1.03), medium for total root length (Trl) (0.4) and low for average root diameter (Ard) (0.27) (Table 2). The highest values of epistatic genetic variance ($\hat{V}_I$) were for Proot in Assay1 (0.3), while Nroots, (0.11 for Assay1 and 0.12 for Assay2), Proot in Assay2 (0.09) and Trl (0.06) presented intermediate values. Finally, Ard and Rv presented low values (0.02) for $\hat{V}_I$ (Table 2; Fig. 3). The phenotypic variance ($\hat{V}_P$) remained around 1 for all continuous variables analysed, because of the standardization of the data. Nroot and Proot showed the highest proportion of genetic variance ($\hat{V}_G$) in both assays with values between

0.33 and 0.4; the root quality variables showed lower $\hat{V}_G$ with values between 0.07 and 0.17 (Table 2).

The narrow-sense heritability ($\hat{h}^2$) was low for all the variables, with the highest estimate for number of roots (Nroots) in Assay1 (0.1), followed by total root length (Trl) (0.08), root volume (Rv) (0.07), the presence of roots (Proot) in Assay2 (0.07), Nroots in Assay2 (0.07), Proot in Assay1 (0.06) and average root diameter (Ard) (0.06). The broad-sense heritability ($\hat{H}^2$) values were much higher: the highest values were 0.43 and 0.35 for Nroots in Assay1 and Assay2, followed by 0.33 for Proot in both assays; Trl and Rv showed intermediate estimates (0.17 and 0.15, respectively), and Ard showed the lowest (0.09). The highest estimates of $\hat{H}^2$ were for Proot and Nroots (Assay1 and Assay2) because of the contribution of the non-additive components of the genetic variance. Finally, the highest value of the fraction $\hat{h}^2/\hat{H}^2$ was 0.61 for Ard; the lowest values were 0.21 for Proot (Assay1 and Assay2), 0.23 (Assay1) and 0.17 (Assay2) for Nroots, while Trl and Rv had intermediate values around 0.5.

Considering the breeding values of the female and male parents together, the parents with the highest values for the presence of roots (Proot) were those with ancestry in *C. crenata* and two of the Galician traditional varieties of *C. sativa*, and the parents with the lowest values were some of the *C. sativa* parents (Fig. S1, A and B). With respect to the root system quality variables, the parents with the highest values were *C. mollissima* and most of the *C. sativa* parents (Fig. S1, C–E). The families with the highest and the lowest values varied according to the analysed variable, without following any concrete pattern of parent combination (Fig. S2, A–C). Figure 4 shows that there were individuals within all families with high breeding values in Assay2 for the variables Proot (Fig. 4A), Nroots (Fig. 4B) and Trl (Fig. 4C), even within families with the lowest breeding values.

Analysis with Model2

The results of the analysis with Model2 of data from 16 families obtained in 2010 and rooted for two consecutive years were similar to those obtained with Model1 for Assay1 (Table S1). For the variable Proot, the variance of GCA and I(FM) remained significant but with lower variances than in analysis with Model1; consequently $\hat{h}^2$ was practically 0 and the value of $\hat{H}^2$ remained moderate (0.25) because of epistasis. For the variable Nroots, the variances due to SCA and I(FM) remained significant and the $\hat{H}^2$ value also remained also moderate (0.37) due, in this case, to dominance.

Correlations between rooting characters and the evolution of rooting ability with age

The phenotypic correlation values estimated in Assay2 between the presence of roots (Proot) and number of roots (Nroots) and between Nroots and total root length (Trl) or root volume (Rv) were moderate (0.46, 0.42 and 0.36, respectively), while there were no correlations between Proot and other variables or between Nroots and average root diameter (Ard; 0.03). Trl had a very high correlation with Rv (0.83) but not with the variable Ard (0.01). Finally, the correlation between Ard and Rv was low (0.26). Scatter plots of standardized measurements of the traits between Nroots and Trl and between Nroots and Rv, both estimated in Assay2, are shown in Fig. S3, A and B.

The genetic correlations of dominance and additive components are not shown for all cases because at least one of the variables was not significant for SCA or GCA, respectively. In Assay2, the type A genetic correlation between the presence of roots (Proot) and number of roots (Nroots) was very high (0.93, Fig. 5A), while there were no correlations with the rest of the variables. Conversely, the type A genetic correlations for Nroots with total root length (Trl) and root volume (Rv) were lower than the phenotypic correlations (0.28 and 0.28, respectively; Fig. 5B and C), while the correlation with average root diameter (Ard) was low and negative (−0.2). Trl had a very high correlation with Rv (0.88) with a moderate additive component (0.48) but not with the variable Ard (0.07). Finally, the correlation between Ard and Rv was higher than the phenotypic correlation (0.37) and had a high additive component (0.78). However, these results should be interpreted with caution because

of the high standard errors obtained. The breeding values of the individuals for each variable were used to generate the graphs in Figure 5.

The type B genetic correlations obtained with Model2 were calculated for the presence of roots (Proot) and number of roots (Nroots) between Assays 1 and 2, because they were the only common variables evaluated in both assays. The dominance correlation for Proot is not shown because one of the variables was not significant for SCA, while the additive correlation for Nroots is not shown because one of the variables was not significant for GCA. The estimated genetic correlation was high for both variables (0.78–0.79), with a strong additive component for Proot (0.76) and a strong dominance component for Nroots (0.75). Because the standard errors were low, these estimates can be considered reliable.

Discussion

Good rooting at the juvenile stage

The rooting percentages obtained in the two trials presented in this paper (91% and 82%) are much higher than those obtained several decades ago, in worse facilities, of around 40% with high levels of mortality (Urquijo 1952; Graves and Nienstaedt. 1953; Viéitez et al. 1999). They are also higher than those obtained in previous trials performed under similar technical conditions with rooting percentages of 22–45% (Fernández-López et al. 1992), 42–95% (Ocaña et al. 2001), 1.23–84% (Rodríguez et al. 2005) and 7–53% (Dantas et al. 2016) for old clones. They are also higher than the rooting percentages of control old clones (60 years) in Assays1 and 2 with mean values by clone of 64%. Additionally, the numbers of roots obtained in our study (mean values of 18 in Assay1 and 10 in Assay2) were also higher than the values of the control old clone 2522 (a mean of 6 roots) and those obtained in another trial with values of 1.5–3.6 (Dantas et al. 2016).

The improved rooting percentage in our assays can be attributed mainly to the juvenility of the materials we used. It is a known fact that maturation leads to a decrease in rooting capacity (Foster

et al. 1981; Greenwood and Hutchinson 1992; Giovannelli and Giannini 2000; Makouanzi et al. 2014). However, to determine how rooting capacity decreases with time, it is necessary to perform assays over a number of years, using the same mother plants as starting materials for cuttings. Considering that some old clones (60 years old) still maintain good rooting capacity (e.g. control clone 103 with an 87% rooting percentage), we expect that, at least, some young clones with good rooting selected in this experiment will maintain their rooting capacity for a long time. We have some information about the decrease of rooting percentage from a set of 60 ortets selected within 10 of the families obtained in 2010 and preselected as potential rootstocks. All of these materials showed 100% rooting in Assays 1 and 2; after a new propagation in 2015, at five years old, the rooting percentage decreased to 85% and only 23 ortets showed 100% rooting. Consequently, it is probable that the decrease in rooting, due to the maturation of clones, is a rapid process.

Rooting in the juvenile state is under moderate genetic control due to non-additive genetic components

We consider the results of Assay2 to be more valid than those of Assay1 because Assay2 included more parents, families and individuals; the increase in the number of parents in Assay2 resulted in an increase in the GCA effect, which became close to significant for Nroots (Table 2). We can therefore say that GCA, SCA and the variance of individuals within families are significantly different from zero for the presence of roots and for the number of roots. Regarding causal factors of genetic variance, dominance was much higher than the additive genetic variance ($V_D/V_A = 2.43$ and 2.83). These results are very similar to those obtained with hybrids of the genus *Eucalyptus* (Makouanzi et al., 2014). However, it cannot be generalized that $V_D > V_A$ in the rooting of cuttings for all tree species, because other authors have found contrary trends in *P. taeda* (Foster, 1990 and Baltunis et al., 2005). The epistatic variance in this *Castanea* experiment was also higher than the additive genetic variance ($V_I/V_A = 1.28$ and 2) that Baltunis et al. (2005) observed in their study on *P. taeda*.

The very low values of $\hat{h}^2$ (< 0.10 for all the variables) indicate that little improvement could be achieved by selecting parents that confer greater rooting capacity to their progenies, although some parents showed good GCA for rooting, including some *C. crenata* lines, *C. sativa* (Garrida and Parede) and some interspecific hybrids (2522 and CA-15). However, the values of $\hat{H}^2$ (0.33 to 0.43) for rooting and the number of roots indicate moderate genetic control of the most important rooting traits. The fraction $\hat{h}^2/\hat{H}^2$, with values 0.17–0.21, suggested, once more, that the presence of roots (Proot) and the number of roots (Nroots) had high non-additive genetic variance. Consequently, the selection of families with good rooting, such as some $F_1(Cc \times Cs)$ and $F_2(Cc \times Cs)$ families, or the selection of individuals within families, will provide a significant improvement in rooting capacity. Small values of $\hat{h}^2$ were also found in previous studies of *Eucalyptus* (Makouanzi et al., 2014) and *P. taeda* (Foster, 1990 and Baltunis et al., 2005), and the $\hat{H}^2$ values were generally lower than those obtained in our trials with chestnut. We conclude that adventitious root formation is under moderate genetic control due to non-additive genetic variance. With respect to the variables of root system quality, the values of the fraction $\hat{h}^2/\hat{H}^2$ were higher (0.44–0.61) than for rooting and the number of roots. This result suggests that these characteristics are under weak genetic control, because they were lower than the values obtained for the number of roots.

In our study, $\hat{V}_I$ is confused with ‘C effects’ because the ramets of each clone were derived from the ortet of the initial plant as in Baltunis et al. (2005).

Correlation among rooting traits and among experiments ($\hat{r}_G$)

The high genetic correlation between the presence of roots and the number of roots ($\hat{r}_G = 0.93$; Fig. 5A) confirms that both variables are part of the same process and are controlled by the same genes. This high correlation assures that the selection of families and individuals with good rooting percentages will lead to an improvement in the number of roots.

Conversely, the reduced or zero values for genetic correlation between the formation of adventitious roots and their later development indicate that these processes are controlled by different genes. This is an opposite result to that obtained in other species (Gravel-Grenier et al., 2011).

For rooted cuttings to have good later development, their root systems need to be of a certain quality, which can be defined by a minimum number of roots or a minimum root length or volume. We know that the number of roots is a very important variable to define the quality of the root system because it is highly correlated with plant survival in the later nursery stage ($r = 0.47$, $P < 0.0001$; Míguez et al., 2016). Conversely, we do not know how important the other parameters of the root system architecture are for the survival and later development of plants and their adaptation to drought, although we assume that, for example, the volume of the root system must be very important. The absence or low genetic correlation between the emergence of adventitious roots and other characteristics of the root system indicates that it is necessary to select independently for each target characteristic. Despite the low genetic correlation, it is possible to select clones with good characteristics for root number and volume or length of roots (Fig. 5B and C).

Other considerations

Considering these data together with those obtained in other experiments, we can begin to predict how to select for several relevant traits in chestnut breeding programmes. The relevance of the additive and dominance components of the genetic variance for flushing (Míguez et al., 2016) and resistance to *P. cinnamomi* (data not published) indicate that the highest gains can be obtained by selecting parents and families with good characteristics. From the data obtained in the current research for adventitious root formation in cuttings, we can conclude that clones with good rooting can be selected within families with resistance to *Phytophthora* sp. and late flushing.

Both experiments described here (Assay1 and Assay2) were conducted in the spring with new shoots grown from small stumps. After cutting that first harvest, the stumps produce new shoots

from the buds formed at their bases and these new shoots can be rooted again as in other species such as *P. taeda* (Baltunis et al. 2005) and *Eucalyptus* spp. hybrids (Makouanzi et al. 2014).

Conclusions

Although the species of the genus *Castanea* are recalcitrant to rooting by cuttings, the use of softwood cuttings, taken from the base of pruned plants, the application of IBA and rooting in fog-system environments allow to the rooting of cuttings to be used as a technique for plant production. As most old clones show poor rooting, we intend to improve rooting percentages by selecting good rooting clones at a young age. One of the conditions for effective selection is that rooting is under genetic control. We demonstrated that the rooting of chestnut cuttings is subject to moderate genetic control due mainly to non-additive genetic variance. Consequently, it is important to consider the rooting capacity among the selection traits at a young age.

The low importance of additive genetic variance implies that, in our chestnut breeding programme, there is no reason to reject parents chosen for other characteristics such as late flushing or resistance to *Phytophthora*. Additionally, the results demonstrate the importance of the dominance component of the genetic variance and, consequently, the possibility of selecting good rooting families. However, we could also select good rooting clones within each family. We expect that the selection of good rooting clones in the juvenile state will lead to good rooting of mature clones in future or, at least, clones with better rooting than if this characteristic is not considered among the selection traits.

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Table 1. Families tested by their rooting ability, their genealogical classes and the numbers of individuals of each family employed in assays 1 and 2; (A) pollinations conducted in 2010, (B) pollinations conducted in 2011.

A) Pollinations 2010					B) Pollinations 2011			
♀	♂	Genealogical Classes	Individuals Assay1	Individuals Assay2	♀	♂	Genealogical Classes	Individuals Assay2
2522	23M09	BC(CcxCs)Cc	21	18	2522	19-C-17	BC(CcxCs)Cs	17
2522	M5M	(CcxCs)Cm	20	18	2522	21-LU-29	BC(CcxCs)Cs	17
CA-15	23M09	BC(CcxCs)Cc	12	12	2522	5-LU-29	BC(CcxCs)Cs	15
CA-15	2522	F2(CcxCs)	25	23	CA-15	19-C-17	BC(CcxCs)Cs	18
CA-15	M5M	(CcxCs)Cm	7	7	CA-15	21-LU-29	BC(CcxCs)Cs	18
Garrida1a	23M09	F1(CsxCc)	20	20	CA-15	5-LU-29	BC(CcxCs)Cs	6
Garrida1a	2522	BC(CcxCs)Cs	22	20	Parede1	21-LU-29	CsxCs	16
Garrida1a	CA-15	BC(CcxCs)Cs	11	11	Parede1	5-LU-29	CsxCs	20
Parede1	Presa	CsxCs	17	10	Parede1	19-C-17	CsxCs	16
Parede1	23M09	F1(CsxCc)	27	18	Total			153
Parede1	2522	BC(CcxCs)Cs	24	22				
Parede1	CA-15	BC(CcxCs)Cs	18	13				
Parede1	M5M	F1(CsxCm)	21	21				
Parede2b	2522	BC(CcxCs)Cs	26	22				
Presa	23M09	F1(CsxCc)	13	13				
Presa	2522	BC(CcxCs)Cs	10	10				
Total			294	258				

Cs: *Castanea sativa*, Cc: *Castanea crenata*, Cm: *Castanea mollissima*, BC: backcross, F1: first generation, F2: second generation.

Table 2. Overall means ($\bar{X}$) and ranges (in brackets), estimates of variance components (significance based on likelihood ratio tests and standard errors) and estimates of genetic parameters (and standard errors) for the measured traits based on the MIXED procedure for continuous variables and the GLIMMIX procedure for presence of root with a binomial distribution and a logic link function for binary traits using model1. Proot = presence of root (Assay1 and Assay2), Nroots = number of roots (Assay1 and Assay2), Trl = total root length (Assay2), Ard = average root diameter (Assay2) and Rv = root volume (Assay2).

	Proot (Assay1)	Proot (Assay2)	Nroots (Assay1)	Nroots (Assay2)	Trl (Assay2)	Ard (Assay2)	Rv (Assay2)
$\bar{X}$	0.91 (0 – 1)	0.82 (0 – 1)	17.93 (1 – 73)	10 (1 – 76)	250.56 cm (5.3 – 654.23)	1.18 mm (0.79 – 1.74)	2.92 cm ³ (0.82 – 7.36)
σ_{GCA}^2	0.014***	0.018**	0.024 ^{ns}	0.015 ^{ns}	0.02**	0.014***	0.018**
σ_{SCA}^2	0 ^{ns}	0.043***	0.055**	0.041**	0.008 ^{ns}	0.004 ^{ns}	0.017 ^{ns}
$\sigma_{I(FM)}^2$	0.30***	0.26***	0.325***	0.275***	0.122***	0.06***	0.104***
$\hat{V}_A$	0.06±0.23	0.07±0.28	0.10±0.10	0.06±0.04	0.08±0.05	0.06±0.03	0.07±0.04
$\hat{V}_D$	0	0.17±0.59	0.22±0.16	0.16±0.10	0.03±0.04	0.02±0.03	0.07±0.05
$\hat{V}_I$	0.30±0.15	0.09±-0.39	0.11±-0.13	0.12±-0.07	0.06±-0.03	0.02±-0.02	0.02±-0.04
$\hat{V}_D/\hat{V}_A$	0	2.43	2.26	2.83	0.4	0.27	1.03
$\hat{V}_I/\hat{V}_A$	4.68	1.28	1.1	2	0.75	0.33	0.29
$\hat{V}_P$	1.01±0.41	1.01±0.51	0.99±0.14	1.0±0.10	1.0±0.09	1.01±0.08	1.02±0.09
$\hat{V}_G$	0.33±0.38	0.34±0.47	0.43±0.12	0.35±0.08	0.17±0.06	0.09±0.04	0.16±0.06
$\hat{h}^2$	0.06±0.05	0.07±0.06	0.10±0.10	0.06±0.04	0.08±0.05	0.06±0.03	0.07±0.04
$\hat{H}^2$	0.33±0.06	0.33±0.06	0.43±0.07	0.35±0.04	0.17±0.03	0.09±0.03	0.15±0.03
$\hat{h}^2/\hat{H}^2$	0.21	0.21	0.23	0.17	0.47	0.61	0.44

σ_{GCA}^2 = General combining ability, σ_{SCA}^2 = Specific combining ability, $\sigma_{I(FM)}^2$ = Individual within the full-sib family, $\hat{V}_A$ = additive, $\hat{V}_D$ = Dominance, $\hat{V}_I$ = Epistatic, $\hat{V}_G$ = Genetic, $\hat{V}_P$ = Phenotypic $\hat{h}^2$ = Individual tree narrow sense, $\hat{H}^2$ = Individual tree broad sense. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} not significant. The values of the variances of the variable presence of root (Proot) were standardized to 1 in order to compare them with the values of the other variables.

Figure 1. Example of an initial plant used as a stock mother plant for cuttings of the family Parede1×23M09 (individual 14). The plant was coppiced at 4–5 cm in January and the photo was taken at the point when the cuttings were cropped for rooting.

Figure 2. Rooting differences between individuals of different families from controlled pollinations in Assay2. A. Family Parede1×21-LU-29, individual 41; B. Family Parede1×5-LU-29, individual 110; C. Family Parede1×5-LU-29, individual 11 and Family Parede1×2522, individual 46.

Figure 3. Proportion of variance components (white = $\hat{V}_A$, gray = $\hat{V}_D$ and black = $\hat{V}_I$) of the seven variables (presence of root (Proot), number of roots (Nroots), total root length (Trl), average root diameter (Ard) and root volume (Rv)). The values of the variances of the variable presence of root (Proot) were standardized to 1 in order to compare them with the values of the other variables.

Figure 4. Breeding values of individuals for the measured variables based on the best linear unbiased predictor methodology, in the Assay2: A. Proot = presence of root; B. Nroots = number of roots; C. Trl = total root length. The names located on the horizontal axis are family codes.

Figure 5. Relationships between the breeding values of individuals in Assay2 for (A) Nroots = number of roots and Proot = presence of root ($\hat{r}_G = 0.93$); (B) Nroots = number of roots and Trl = total root length and between; (C) Nroots = number of roots and Rv = root volume.

Table S1. Overall means ($\bar{X}$) and ranges (in brackets), estimates of variance components (significance based on likelihood ratio tests and standard errors) and estimates of genetic parameters (and standard errors) for the measured traits based on the MIXED procedure for continuous variables and the GLIMMIX procedure for presence of root with a binomial distribution and a logit link function for binary traits using model2. Proot = presence of root (Assay1 and Assay2), Nroots = number of roots (Assay1 and Assay2), Trl = total root length (Assay2), Ard = average root diameter (Assay2) and Rv = root volume (Assay2).

	Proot (Assay1 and Assay2)	Nroots (Assay1 and Assay2)
$\bar{X}$	0.84 (0 – 1)	13.34 (1 – 76)
σ_{GCA}^2	0.002*	0.013 ^{ns}
σ_{SCA}^2	0 ^{ns}	0.115**
$\sigma_{I(FM)}^2$	0.243***	0.2***
$\sigma_{GCA}^2 * a$	0.0002 ^{ns}	0.003 ^{ns}
$\sigma_{SCA}^2 * a$	0.098**	0.04*
$\sigma_{I(FM)}^2 * a$	0.484***	0.042**
$\hat{V}_A$	0.009±0.064	0.05±0.096
$\hat{V}_D$	0	0.459±0.307
$\hat{V}_I$	0.243±0.194	0.144±0.246
$\hat{V}_D/\hat{V}_A$	0	9.18
$\hat{V}_I/\hat{V}_A$	27	2.88
$\hat{V}_P$	1.002±0.292	0.988±0.19
$\hat{V}_G$	0.248±0.258	0.34±0.156
$\hat{h}^2$	0.009±0.149	0.051±0.228
$\hat{H}^2$	0.251±0.078	0.37±0.289
$\hat{h}^2/\hat{H}^2$	0.036	0.137

σ_{GCA}^2 = General combining ability, σ_{SCA}^2 = Specific combining ability, $\sigma_{I(FM)}^2$ = Individual within the full-sib family, $\hat{V}_A$ = additive, $\hat{V}_D$ = Dominance, $\hat{V}_I$ = Epistatic, $\hat{V}_G$ = Genetic, $\hat{V}_P$ = Phenotypic $\hat{h}^2$ = Individual tree narrow sense, $\hat{H}^2$ = Individual tree broad sense. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} not significant. The values of the variances of the variable presence of root (Proot) were standardized to 1 in order to compare them with the values of the other variables.

Figure S1. Breeding values of the parents for the measured variables based on the best linear unbiased predictor methodology: A and B. Proot = presence of root (Assay1 and Assay2); C. Trl = total root length (Assay2); D. Ard = average root diameter (Assay2); D. Rv = root volume (Assay2). The names located on the vertical axis are the parent codes used in controlled pollinations.

Figure S2. Breeding values of families for the variables Proots (presence of root) and Nroots (number of roots) based on the best linear unbiased predictor methodology: A. Proot (Assay2); B. Nroots (Assay1); C. Nroots (Assay2). The names located on the vertical axis are the family codes.

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Figure S3. Relationship between the standardized values of (A) Nroots = number of roots and Trl = total root length ($\hat{r}_p = 0.42$) and between (B) Nroots = number of roots and Rv = root volume ($\hat{r}_p = 0.36$) in Assay2.

Table S1. Overall means ($\bar{X}$) and ranges (in brackets), estimates of variance components (significance based on likelihood ratio tests and standard errors) and estimates of genetic parameters (and standard errors) for the measured traits based on the MIXED procedure for continuous variables and the GLIMMIX procedure for presence of root with a binomial distribution and a logit link function for binary traits using model2. Proot = presence of root (Assay1 and Assay2), Nroots = number of roots (Assay1 and Assay2), Trl = total root length (Assay2), Ard = average root diameter (Assay2) and Rv = root volume (Assay2).

	Proot (Assay1 and Assay2)	Nroots (Assay1 and Assay2)
$\bar{X}$	0.84 (0 – 1)	13.34 (1 – 76)
σ_{GCA}^2	0.002*	0.013 ^{ns}
σ_{SCA}^2	0 ^{ns}	0.115**
$\sigma_{I(FM)}^2$	0.243***	0.2***
$\sigma_{GCA}^2 * a$	0.0002 ^{ns}	0.003 ^{ns}
$\sigma_{SCA}^2 * a$	0.098**	0.04*
$\sigma_{I(FM)}^2 * a$	0.484***	0.042**
$\hat{V}_A$	0.009±0.064	0.05±0.096
$\hat{V}_D$	0	0.459±0.307
$\hat{V}_I$	0.243±0.194	0.144±0.246
$\hat{V}_D/\hat{V}_A$	0	9.18
$\hat{V}_I/\hat{V}_A$	27	2.88
$\hat{V}_P$	1.002±0.292	0.988±0.19
$\hat{V}_G$	0.248±0.258	0.34±0.156
$\hat{h}^2$	0.009±0.149	0.051±0.228
$\hat{H}^2$	0.251±0.078	0.37±0.289
$\hat{h}^2/\hat{H}^2$	0.036	0.137

σ_{GCA}^2 = General combining ability, σ_{SCA}^2 = Specific combining ability, $\sigma_{I(FM)}^2$ = Individual within the full-sib family, $\hat{V}_A$ = additive, $\hat{V}_D$ = Dominance, $\hat{V}_I$ = Epistatic, $\hat{V}_G$ = Genetic, $\hat{V}_P$ = Phenotypic $\hat{h}^2$ = Individual tree narrow sense, $\hat{H}^2$ = Individual tree broad sense. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} not significant. The values of the variances of the variable presence of root (Proot) were standardized to 1 in order to compare them with the values of the other variables.

Figure S1. Breeding values of the parents for the measured variables based on the best linear unbiased predictor methodology: A and B. Proot = presence of root (Assay1 and Assay2); C. Trl = total root length (Assay2); D. Ard = average root diameter (Assay2); D. Rv = root volume (Assay2). The names located on the vertical axis are the parent codes used in controlled pollinations.

Figure S2. Breeding values of families for the variables Proots (presence of root) and Nroots (number of roots) based on the best linear unbiased predictor methodology: A. Proot (Assay2); B. Nroots (Assay1); C. Nroots (Assay2). The names located on the vertical axis are the family codes.

Draft

Figure S3. Relationship between the standardized values of (A) Nroots = number of roots and Trl = total root length ($\hat{r}_p = 0.42$) and between (B) Nroots = number of roots and Rv = root volume ($\hat{r}_p = 0.36$) in Assay2.