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Project 4330

ADVANCED AND CONVENTIONAL METHODS FOR VEGETATIVE PROPAGATION OF SELECTED LINES OF OAK AND CHERRY

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ADVANCED AND CONVENTIONAL METHODS FOR VEGETATIVE PROPAGATION OF SELECTED LINES OF OAK AND CHERRY

Project 4330

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CONTENTS

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Summary

Oak and wild cherry (*Prunus avium*) are important broadleaved species. We have identified superior trees and conserved them by grafting. Methods for the large scale propagation of selected trees was evaluated using the technology of micropropagation. For oak we could establish shoot cultures from mature oaks but their propagation rate was too low and difficult for practical application. With wild cherry micropropagation is a feasible option for large scale production of selected material.

Genetic fingerprinting technology was developed and applied to oaks. We showed that all material propagated vegetatively from oak was genetically similar to the original donor tree. Furthermore we used genetic fingerprinting to analyse a unique stand of elite oaks in Co. Westmeath and determined that the selected trees were not closely related to each other. The analysis of genetic fingerprints of 16 elite oaks also showed that five of them had an unusual pattern of DNA and one tree was identified as a rare triploid tree..

Introduction.

Oak (*Quercus robur and Quercus petraea*) and wild cherry *(Prunus avium*) are important hardwoods in Ireland. The planting stocks available today are similar in their genetic constitution to the material in native woodlands of 8000 years ago. There has been no practice of genetic improvement for these species over the years. Genetic improvement of trees is a long term process because of the need to evaluate the progeny over several decades. However, modern methods of rapid propagation of selected trees can speed up several stages in the process of tree improvement by making material available for testing more rapidly. A programme of improvement requires that only the most superior trees in natural populations are selected and used. The germplasm of selected trees is conserved by vegetatively propagating from them. These mother trees become the foundation stock on which genetic selections are made. Furthermore, it is feasible to use the selected trees directly in the improvement programme provided they can be propagated by vegetative means in an efficient manner on a large scale. In this way a collection of elite mother trees could form the basis for improved varieties. An essential step in this scheme is vegetative propagation of selected material.

Mature trees have undergone an ageing process and because of ageing they are physiologically different to seedlings. Generally the mature phase is characterised by seed formation and also by a low capacity of cuttings from them to produce roots. This project describes different systems for vegetatively propagating oak and cherry and evaluates the effectiveness of these methods for accelerating an improvement programme. In addition we have used genetic fingerprinting technology to characterise selected oaks at different stages of propagation.

Materials and Methods

Approximately 100 elite trees of oak (*Quercus robur* and *Q. petraea*) and cherry (*Prunus avium*) were identified by foresters in Coillte throughout their estates. Scion wood was collected by climbing trees. Such elite trees are defined as "clean, with straight stems and butts, free from knots, as round as possible with the heart straight down the middle, free from wandering heart, with minimal taper and without star or ring shape or epicormic burrs".

Buds of oak and cherry were selected at the beginning of the growing season, surface sterilized and cultured in vitro. For oak the medium was WPM with BA 0.2mg/L (Lloyd and McCown 1980). For cherry, MS basal medium (Murashige and Skoog, 1962) medium was used containing 1.0mg/L BA, (benzyladenine) 1.0 mg/L IBA (indolebutyric acid) and 0.1 mg/l GA3 (gibberellic acid). Cultures were maintained at 22^0C with .16 hr. photoperiod 40w-32µE $m^{-2}.sec^{-1}$ and were transferred to fresh medium every 28-32 days. Other methods are described throughout the text.

Results and Discussion

<u>Grafting oak and cherry</u>.

Scions of oak and cherry were cleft grafted in March to 2 yr. old rootstocks. For cherry we obtained an overall graft viability of 40% and 85% of the individual trees selected were successfully grafted. For oaks the graft viability ranged from 10 to 17% per year and 64% of all clones grafted were successfully propagated.

An alternative method of summer grafting was developed for oak. It used 4-8 month old rootstocks and scions collected from plants grafted conventionally in the previous year. A diagonal cut of 50^o was made to the stock and scion selected with a stem diameter of 3.3mm. Laboratory silicone tubing (internal diameter 3.2mm, wall thickness 1.6mm) was cut into lengths of 2 to 3 cm and first placed halfway over the stock. By viewing through the wall of the tube already on the stock, the scion was

pushed into the tube so that its cut surface was guided across and matched to the cut surface of the stock (Fig 1). Scions generally consisted of a single lignified node 3 cm in length from which the leaf was excised. All buds on the stock were also excised using a scalpel. Grafts were tied to a small stake, covered with a plastic bag which was also tied, and placed in a plastic enclosure in a shaded greenhouse. When scion buds grew out, humidity in the bag was reduced gradually by opening the bag; the wall of the silicone tube was cut open using a scalpel when the bud grew out.

Viable grafts were obtained using a diagonal cut, whereas horizontal cuts failed. Outgrowth of grafted buds occurred in 20 days. Scions consisting of an apical bud, with either 1,2, or 3 axillary buds each gave 50% graft viability, whereas single and double node scions each gave 60% viability (10 to 15 grafts per treatment). Stock plants in which the growth flush had finished gave 50% graft viability, whereas those in which the flush was in progress gave 25% (12 plants per treatment). Using seedlings, variations on the grafting methodology were tested. Single nodes were used as scions from 8 month old unflushed oak and they were either self grafted (autografted) or grafted to a neighbouring plant (heterografted). The effects of delaying the joining of the cut surfaces in the graft by 10 seconds, 1, 5, and 10 minutes was tested. In each treatment 15 plants were grafted and viability was 100% in all treatments . There were no adverse effects of delaying graft joining or grafting plants to neighbours. Similarly 'tight' fits and 'normal' fits were made by selecting stock/scions of appropriate thickness for the silicone tubing and these treatments had no effect on graft viability.

The tube method was tested using mature clones of oak, already established from 2-year-old winter grafts. The single node scions were prepared by first removing the shoot apex between 4 and 7 days before excising the selected frist node (N1) or second ode (N2). Grafting was in August. Control graft were seedling scions heterografted and viability was 68%. Unlike conventional winter grafting, all mature clones grafted were viable: two gave 100% viability while five out of the remaining six gave more than 30% (Fig.2, 10 grafts per clone).
Eight elite trees of *Q. petraea* at Tullynally Castle, Co Westmeath provided scions from crown and epicormic shoots. Using the tube method in August 1995, crown shoots gave 55% viable grafts from one clone and 5% for another. The mean viability of grafting epicormic shoots was 7% with these eight clones (range 0 to 15, 55 grafts per clone) and three clones failed to give viable grafts.

Shoots from cherry roots

Root suckers were examined as a source of buds to initiate cultures of cherry to save the inconvenience of climbing mature trees and also because such suckers are vigorous and may show juvenile characteristics. Roots were excised by excavating soil and carefully using a saw. They were cut into lengths of 20-30cm, washed, dipped in zineb (2.5g/l), placed in a 50.50 mixture of peat and perlite and placed into trays and then into large plastic bags in the greenhouse in a 16 hr photoperiod.

The capacity of different selected trees to produce root suckers varied generally with 8 selected trees failing to give any shoots from roots, Table 1. In general, those trees which gave shoots in 100% of root segments also gave the highest number of shoots per 100 cm of root. We determined that the season of root collection had no clear influence on the capacity of roots to produce shoots. For example in one year, autumn collection of roots gave best responses with three selected trees whereas spring collection was best for three others.

Initiation of cherry cultures

Cultures were initiated from mature and young trees and the effects of three sources of explants on sterility and the percentage of viable buds obtained with several clones is summarised in Table 2. With mature trees the use of root suckers as a source of buds for culture initiation was superior to forced shoots. Root suckers gave double the percentage of buds initially sterile. In addition, for root derived buds,18% remained viable and proliferated whereas only 2% of buds from the crowns of trees remained viable. Root suckers were also superior in terms of the establishment of selected clones in culture with 67% of clones giving proliferating cultures from root suckers and 40% from forced shoots. The rate of clone establishment was also high from young trees(80%). Buds from young trees had a high level of bud sterility and viability.

Micropropagation and rooting of cherry and oak

A viable system of micropropagating mature trees should result in plants which behave as regular seedlings in the field. It requires that either juvenile tissues within mature trees are used e.g. root suckers or epicormic shoots or that the tissues selected can undergo a physiological reversal of the ageing process after several periods of culturing. We therefore observed both the propagation rate and the growth responses of shoots during their propagation.

The micropropagation coefficient (number of explants obtained per viable explant) was obtained for several selections of cherry and is summarised in Fig 3. Shoot production varied throughout the period with each selected tree. Rooting capacity was over 80% with most elite clones and all plants which were rooted in vitro were successfully weaned to the glasshouse. In this way we produced 300 plants of each of three selected trees of wild cherry . The plants were subsequently planted in the field for further evaluation of their growth potential.

Cultures were established from mature and juvenile trees of oak (Fig. 4). The micropropagation coefficient was generally 1 to 2.5 for a range of selected material .There was no significant difference in shoot production between juvenile cultures and those derived from selected elite trees in the age group of 80-200 years (Table 3). Cultures from mature trees never showed evidence of a juvenile growth (vigour and vertical growth of shoots) even after several years of culturing. This may indicate that the culturing in vitro failed to rejuvenate oak buds from mature trees. The rooting capacity of mature oak shoots in culture rarely exceeded 40% and weaning to the glasshouse was difficult. Micropropagation of oak is not a feasible option to provide material derived from mature trees. In addition, the oak cultures failed to show any signs of rejuvenation ie straight growth.

We examined cultures derived from seedlings and those derived from mature plants in efforts to characterise the morphological characteristics associated with the phases of juvenility and maturity respectively. This analysis could provide a basis for detecting a shift from the mature patterns of growth to more juvenile characteristics which can occur in vitro after periods of prolonged culturing. This physiological shift is necessary for trees that are produced from mature trees because only juvenile trees or rejuvenated material will perform well under field conditions. Rejuvenation techniques which are available for the re-invigoration of oak shoots rely on the availability of good quantitative markers for juvenile and mature plants.

To provide such markers, shoot cultures of *Quercus* species of juvenile, adolescent and mature origin were examined for a range of morphological and physiological markers of juvenility and maturity *in vitro*. Criteria examined were angle of the shoot to the horizontal, stem length, stem diameter (tip, mid, base), leaf number, scale leaf number and shoot number. Image analysis was also carried out to determine leaf area, size, and breadth and length of leaves. Mature *Q. robur* clones showed a larger mid-stem and tip diameter than juvenile clones, whereas mature *Q. petraea* clones were characterised by larger mid-stem and tip diameters and plagiotropic growth (horizontal stem growth) compared with juvenile material. Based on discriminant analysis of the data, for *Q. robur* and *Q. petraea* we proposed the following formulae for discrimination of juvenile and mature shoots where a negative value for the discriminant score (D) indicates juvenility. SA = stem

angle to the horizontal (°), TD =tip diameter of the shoot (mm) and MSD = mid -shoot diameter (mm).
For *Quercus petraea:* discriminant score D = -1.308 - 0.0351 SA + 2.206 TD + 1.435 MSD
For *Quercus robur:*discriminant score D = -3.546 + 2.418 TD + 2.202 MSD
Q.robur shoot cultures derived from stump sprouts and designated as juvenile had a negative D value suggesting a juvenile status for these clones. Cultures sourced from a hedged, grafted *Q. robur* tree of mature origin had a positive D value indicating a mature status. Clones initiated from a 20-25 year old *Q. petraea* tree displayed a morphology in vitro consistent with a mature status and had a positive D value; however, these two clones displayed other traits such as vigour suggesting that vestiges of juvenility remain. The micropropagation rate and leaf size and shape were variable among clones and did not provide suitable markers for juvenility or maturity in these two species. These results provide useful parameters to determine the maturity status of shoot cultures of oak. They support observations that some tissues such as stump sprouts or epicormic shoots retain a juvenile physiological status. Furthermore, the appearance of mature traits in a 25yr. old tree suggests that the maturation process in oaks may begin earlier than previously thought.

Genetic Fingerprinting

A. Genetic stability during propagation

Genetic fingerprinting is a technique that is based on the unique genetic code of each individual and is based on the pattern of DNA fragments which can be visualised after their separation on a gel. (Figs 4-6). It was applied to oaks to check on the genetic fidelity of cuttings taken from different parts of a mature tree and in shoots propagated from it in vitro. In principle all cells of an organism should have the same DNA. In many plants different tissues have different amounts or configurations of DNA and tissues of old organisms can accumulate mutations. Oaks, almost uniquely, produce buds throughout their life which remain dormant and become buried in the bark as the trunk/branch increases in size. They can be stimulated to grow into shoots by exposure to light and water and are useful for propagation since they retain juvenile traits that are associated with young plants such as a capacity to root.

DNA was isolated from crown, epicormic and *in vitro* leaves originating from a single100 yr.old tree of *Q. petraea* and analysed by randomly amplified polymorphic DNA (RAPD)and microsatellites. The principle of microsatellite analysis is given in Fig. 5. With each random primer a characteristic RAPD pattern was obtained and it was common to all six epicormic shoots derived from different parts of a single branch of this tree and also to the shoots from the crown of the same tree with OPE1 OPA-05, OPA-08, OPA-01, OPA-02, OPA-04, OPA-05, OPG-02, OPG-10, OPE-12. Similarly, the RAPD pattern obtained from cultures *in vitro*, derived from individual nodes of epicormic shoots produced by six different branch segments, were uniform for each of 15 primers. This work was repeated using microsatellite PCR . Three microsatellite loci AG16, AG 1/2 and AG 1/5 were amplified by PCR. It showed a uniformity of these microsatellite loci in shoots from the crown of the tree and from epicormic shoots cultures derived from 6 different sections of branch. Fig 6.

B. Genetic analysis of elite oak trees

A morphological and molecular characterisation of phenotypically elite oaks *Quercus robur* L. which were estimated as 220 years old was undertaken to test the utility of molecular tools to examine the genetic origin and relatedness of trees in a superior stand at Tullynally Castle, Castlepollard, Co.Westmeath. The 11 trees shared many excellent characteristics in tree form. Quantitatively, DBH ranged from 104 to 126.5 cm and stem height from 10 to 25.5 m Table 4. The molecular analysis used microsatellites for nine genetic loci (genes) on five trees. In this case the pattern of DNA can be represented by two main bands for each tree representing different forms of a

gene (alleles). Some trees showed a single form of a gene (e.g. Tree 8 Fig 7); in this case, the father and mother tree passed a single form to the offspring. Although all the eleven trees were of excellent quality we concluded from analysing all the genetic loci that the trees were not closely related genetically and it confirms more recent studies that oak pollen can travel from several kilometres . This small sample showed many polymorphisms and much heterozygosity. Loci AG16 and AG 9 showed 9 and 8 different alleles respectively while loci AG1/2 and Ag15 displayed 3 and 5 alleles among the five trees. At least two trees had a three- band profile for some loci indicating the possibility that some trees may be triploids.
The historical records of the estate refers to one elite tree in 1837 and the detail of its description suggests it may correspond to one extant today. It also suggests an active silvicultural management and the practise of coppicing with standards. Such management may have resulted in this excellent stand by conversion of a natural woodland in stages starting with coppice, leading to coppice with standards, then to high forest and ultimately to the parkland stand of today.

C. Discovery of polyploids by analysing microsatellites

In trees, the presence of extra chromosomes or full sets of chromosomes (polyploids) has been known for a long time. The development of cytological techniques allowed the detection of polyploidy in trees as early as 1927, however these techniques are still very difficult to perform because of the small size of tree chromosomes. Creating or detecting natural triploid or polyploid trees could be valuable in terms of productivity, and as a genetic resource to produce new variability. The development of biochemical tools such as isoenzyme analysis in the sixties and seventies also showed the effects of ploidy changes.

More recently, molecular methods such as AFLPs, RFLPs, RAPDs can now be used to detect or confirm changes in ploidy when used in conjunction with cytological and other morphological traits such as stomata length. An analysis of elite oaks' microsatellites and the size of their stomata showed the occurrence of polyploidy (triploidy / aneuploidy). A confirmed German triploid and diploid oak was used as a controls.

The microsatellite DNA profiles of nine different genes (loci) with 16 elite oaks was carried out. Normally trees have two sources of their genes, one from the father and one from the mother. In the offspring, one sees two forms (alleles) of the gene if the father and mother carried different forms, or a single form if each parent carried a single form. Among elite oaks, eleven trees gave a normal one or two-allelic pattern.Five trees out of 16 displayed a pattern of three forms (tri-allelic pattern) at one or more genetic loci.A tri-allelic pattern was found at 2/9 loci for trees Tullynally 4 and Bree 9, at 3/9 loci for trees Tullynally 11 and Phoenix Park. Two trees gave a tri-allelic pattern for five out of the nine alleles analysed ; one of these was the Irish selection, Dundrum 91, the other was a German tree which was confirmed as having a full extra set of chromosomes (triploid). This suggests that one elite Irish oak was a rare triploid and the others may carry one or more extra chromosomes (aneuploids) .

Previous work by on the confirmed German triploid showed that the length of leaf stomata in a triploid was significantly larger than in diploids (Naujoks *et al*.,1995).This observation holds true for many plant species because of the positive correlation between the amount of DNA in a cell and the size of the cell. It also explains why cells an organisms with higher levels of ploidy have a greater size. Leaves were collected from Irish elite oaks and the underside coated in clear adhesive which was peeled off after drying . The replicas of stomata on the peeled -off adhesive were observed on a slide and measured using a light microscope, a video camera and appropriate

software. For each sample, the stomata in at least three leaves were measured and the means presented are from a random sample of 35 observations per tree.

An illustration of the comparative stomatal sizes is given in Fig.8. The stomata of the triploid were 30.4 μm long while those of the diploid were 22.6 μm. We measured the stomatal lengths of Irish selections and these can be compared to those of a confirmed diploid and a triploid in Table 5. The elite selection, Dundrum 91 which gave a tri- allelic pattern at 5/9 genetic loci also showed large stomata and these were significantly larger than the confirmed diploid ($p < 0.001$). These observations point to the strong possibility that this tree is a triploid. Morphologically, Dundrum 91 is straight, has a DBH of 139.5 cm but is marred by the presence of numerous epicormic shoots; its capacity to produce acorns is not known. Several other elite trees had stomata which were significantly larger than the confirmed diploid; New Ross2 ,Phoenix Park and Bramwald 6, but only some of these were subjected to microsatellite analysis
(Table 5). Confirmation of triploidy could be by counting chromosomes, or by quantifying nuclear chromatin; the latter is in progress for the putative triploid Dundrum 91. For one tree, Mountbellew 26 (Mb26) we observed that the length of stomata in leaves collected from the tree in the field was similar to the length in leaves collected from shoots of this genotype *in vitro*; this indicates the stability of stomata length as a morphological trait (Table 5).

The origin of triploids is probably from female or male gametes which fail to undergo meiotic reduction. Triploid acorns were found to be more frequent in oak trees giving twin seedlings with a high frequency (Johnsson, 1946). Among diploid oaks several individuals were found to produce 2n pollen grains comprising 5-10% of the microspores (Butorina, 1993). If the haploid female gamete carrying a given locus was fertilised by a 2n pollen which was heterozygous for two other alleles, a three-band pattern will appear as the microsatellite profile. Triploids would give a two-band pattern if, for example, the 2n male gamete was homozygous and the egg carried a different allele. Indeed, triploids could give a one-band pattern if each gamete carried the same allele. This may also be the explanation for the presence of a di-allelic pattern in a confirmed triploid at 4/9 genetic loci while the remainder (5/9) were tri-allelic, and similarly for the Irish clone Dundrum 91,which is a putative triploid. An alternative explanation for the tri-allelic pattern at some loci shown in Table 5 is aneuploidy for one or more chromosomes. Indeed hyperaneuploidy and hypoaneuploidy was reported previously for oak in a cytological study (Butorina, 1993).

In practical terms triploidy leads to sterility for many plant species. However, the German triploid described above produced acorns which showed spectacular variability from plant to plant offering the possibility of obtaining novel genetic variation (D. Ewald , personal communication). and the two triploids in Russia were described as having " weak fertility " (Butorina, 1993). This fertility in triploid oak offers possibilities for obtaining a broad spectrum of novel genetic variation among progeny. Such progeny could be valuable in tree improvement programmes for improving productivity, tree form and resistance to biotic elements. In addition, the use of microsatellite markers and stomatal measurements could facilitate experiments to determine the frequency of aneuploids/ triploids in progeny from known triploids. Since triploids or aneuploids have potential to be highly productive, it is feasible to use these tools to screen and select such triploid trees among seedlings or among trees in existing plantations and to compare their performance to diploid trees.

Conclusions

1. Micropropagation is a feasible method for the vegetative propagation of selected elite trees of wild cherry (*Prunus avium)* on a large scale and this method was used to produce plants for field testing.

2. Roots from 74% of elite cherry trees are capable of producing shoots which are a good source of buds to initiate vigorous shoot cultures.

3. Micropropagated cherry displayed juvenile characteristics and could be handled like trees produced from conventional seed.

4. Mature trees of elite oaks (*Quercus robur* and *Q. petraea*) can be micropropagated but the propagation rate is too low for practical purposes. Furthermore mature oaks displayed mature characteristics after vegetative propagation and showed no evidence for a recovery of juvenile morphological characters after prolonged periods of culturing *in vitro*.

5. We showed that mature shoot cultures of oak had some morphological traits which were unique and could be used to distinguish them from juvenile material.

6. Shoots from different parts of mature oak trees (100 years) remained genetically stable and shoots propagated from them were also stable.

7. By analysing elite oaks using microsatellite markers we showed that a group of elite oaks in Westmeath were not closely related genetically and that heiir good form may be due to the management system employed on the estate.
8. Microsatellite analysis of elite oak showed evidence that some trees may have an extra chromosome(s) (aneuploidy) or an extra set of chromomes (triploidy). Trees of higher ploidy were confirmed as having larger stomata. Analysis of microsatellites and stomata is a convenient way of detecting triploids in an oak population. Such trees have greater growth potential and are a source of novel genetic variation for tree improvement.

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Table 1.:The distribution of suckering capacity in root cuttings among 31 elite clones of wild cherry *Prunus avium* and their production of shoots.

			Response of Root Cutting	
Number of clones Tested	**Total length of root cuttings tested (cms)**	**Percentage of root cutting with suckers**	**Mean No of suckers / 100 cm of root (range)**	
14	893	100	24	(4-59)
6	328	50	4	(2-7
2	121	31-33	2	(2-7)
1	10	20-25	8	0
8	558	0	0	0

Table 2. - Effect of tree age and source of bud explants on culture initiation in clones of *P. avium*.

				Response of bud/clones	
Source and type of explants	**Total no. of clones**	**Total no. of buds**	**No. of buds sterile (% sterile)**	**No. of buds viable[z] (% viable)**	**No. of viable clones (% viable)**
Mature trees 40-60 yrs					
Crown buds forced shoot	5	210	36 (17)	4 (2)	2 (40)
Root suckers	15	423	126 (30)	78 (18)	10 (67)
Young trees 6-8 yrs.					
Crown buds not forced	5	70	18 (26)	9 (13)	4 (80)

[z] Viable buds were those which remained viable when sub-cultured and sub-divided to produce shoot clusters.

Table 3 Micropropagation rates (after 16 subcultures) of *Quercus* shoots derived from trees of different developmental ages.

Material Origin	**No. of Explants**	**Propagation Rate Mean no. explant per explant subcultured ± se**
Quercus robur		
Seedling 1	**53**	**2.05 ± 0.12**
Seedling 2	**111**	**1.93 ± 0.06**
Stump Sprout	**132**	**2.28 ± 0.11**
Hedged Stock Plant	**26**	**1.68± 0.19**
Q .petraea		
Seedling 1	**183**	**1.74±0.07**
Seedling 2	**40**	**1.58±0.38**
Adolescent (25 yrs)	**50**	**1.49±0.10**
Mature tree (100yrs)	**40**	**1.67±0.14**

Table 4 . : Characteristics of high quality oaks at Tullynally Castle, Castlepollard, Co Westmeath Ireland . Rating Scale 1= Poorest. 4 = Best.

			……Tree Form Rating 1-4…….				
Tree No.	Stem DBH (cm)	Stem Ht (m)	Buttress form	Stem form	Epicormic shoots	Apical dominance	Total rating
1	104	10.0	4	2	3	2	11
2	149	11.0	2	2	4	2	10
3	108.5	18.5	3	4	2	4	13
4	102.9	16.5	3	3	1	3	10
5	109.7	25.5	2	4	3	4	13
6	103.7	22.0	4	4	1	3	12
7	106.4	20.5	4	2	3	2	11
8	139.5	13.0	2	3	4	4	13
9	114.2	15.0	2	3	1	2	8
10	123.6	11.5	2	3	4	4	13
11	126.5	20.5	2	2	1	4	9

Table 5 : Measurements of stomata length (μm) and allelic patterns in elite oaks and a confirmed triploid; means with the same letter are not significantly different, *Duncan's Multiple range test (shown beside the tree name **A- F**).

Elite Tree Selected	*Stomata significantly different	Triallelic pattern/ no.loci tested nt=not tested	**Mean stomata length (μm) +/- SE**
Tullynally 7	**E, F**	nt	**22.6 +/- 0.31**
Ttullynally 4	**E, F**	2/9	**22.9 +/- 0.47**
Tullynally 11	**F**	3/9	**22.4 +/- 0.30**
Tullynally 6	**E, F**	nt	**23.4 +/- 0.48**
Bree 9	**E, F, D**	nt	**23.5 +/- 0.33**
Thomastown 76	**E, D**	nt	**23.7 +/- 0.36**
MB26 (tree leaves)	**E, D, F**	nt	**23.5 +/- 0.27**
MB26 (*in vitro* leaves)	**E, D**	nt	**23.7 +/- 0.36**
Phoenix Park	**D**	3/9	**24.7 +/- 0.49**
Bramwald 6	**D**	2/9	**24.7 +/- 0.44**
New Ross 2	**C**	nt	**26.4+/- 0.35**
Dundrum 91	**B**	5/9	***28.5 +/-0.44***
135 (German triploid)	**A**	5/9	***30.4 +/- 0.44***
German diploid	**E, F**	nt	***22.6 +/- 0.35***