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Characterization of Filbert (*Corylus*) Species and Cultivars Using Gradient Polyacrylamide Gel Electrophoresis[1]

Z. Ahmad,[2] L.S. Daley,[3] R.A. Menendez,[3] and H.B. Lagerstedt[3]
National Clonal Germplasm Repository
Horticulture Department, Oregon State University,
33447 Peoria Rd., Corvallis, OR 97333

Abstract

A chemical identification procedure previously used to identify apple and pear species, cultivars and clonal accessions, was tried with *Corylus* (filbert, hazel) species, cultivars and clonal accessions. Following electrophoresis, the peroxidase, phenol oxidase, and acid phosphatase isozyme patterns on anionic polyacrylamide gradient gels were determined. These patterns were found to vary between clonal accessions, but did not change, within a given accession during and following the test period (May through October). Thus, these patterns were considered to represent genetic characteristics suitable for identification purposes. The patterns were used to identify 78 *Corylus* accessions at the National Clonal Germplasm Repository Corvallis, Oregon. All accessions tested (species, cultivars and clones) were distinguishable using this system. The diversity of isozyme patterns was greater in *Corylus* than *Pyrus* populations previously sampled. This technique appears to have the potential to readily identify filbert accessions and could be an important aid in the characterization of germplasm material.

Index words: acid phosphatase, *Corylus*, electrophoresis, filbert, hazel, isozyme diversity, peroxidase, plant fingerprinting, phenol oxidase, pear, *Pyrus*

Introduction

Chemical identification (fingerprinting) of plant species and cultivars has received increased attention (3, 4, 6, 7, 20) from plant breeders, the nursery industry, growers, and U.S. trade officials (1) because of the increased recognition of germplasm reserves and the importance of exact clonal identification. Genetic markers are useful in identifying clonally propagated material in many crops (2, 12, 16, 21). The National Clonal Gerplasm Repository (NCGR) system, collects, maintains, identifies and characterizes clones of selected crop genera (11). The NCGR at Corvallis, Oregon is responsible for eight genera, including *Corylus* (filbert, hazel). At present the collection of *Corylus* is much smaller (about 150 accessions, 78 of which are large enough to be sampled) than the world collection of pears (19) which comprises over a thousand accessions. Thus, it was thought possible that this relatively small collection could be identified and classified in one year.

A search of the literature showed that only one electrophoretic fingerprinting method was available for *Corylus* (8), but this method, designed for industrial products, was not appropriate for our purpose. A second method—developed originally for apple cultivars (*Malus*) (13), and then modified for pear (*Pyrus*) accessions (14)—was tested. This second method showed that shoot extracts yielded identifying electrophoretic isozymic patterns. The particular patterns that identified each clone remained constant throughout the test period and were not affected by the age of the plant from which the shoots were collected. This technique can be used with very young specimens before phenotypic morphological characteristics, such as those associated with filbert production, become apparent. This paper reports the first application of this method to the genus *Corylus*.

The gene pool for cultivated plants resides, largely, in the wild or less used species of the same genus, and closely related genera (19). *Corylus* has about nine recognized species (Hummer *et al.*, 1986). In the NCGR collection five species had enough growth to be sampled, these are: *C. avellana*, *C. colurna*, *C. heterophylla*, *C. maxima* and *C. vilmorinii*.

Materials and Methods

Plant material was obtained from the NCGR, Corvallis, Oregon *Corylus* Collection. One-year-old shoots (except when otherwise indicated) were collected from May through

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[2]Senior Scientific Officer, National Agricultural Research Centre, P.O. N.I.H. Islamabad, Pakistan.

[3]Assistant Professor of Horticulture, Research Associate, and Research Horticulturist ARS/USDA, National Clonal Germplasm Repository, Department of Horticulture, Oregon State University, Corvallis, OR 97331.

August 1986. The protein extraction technique used for *Corylus* is the same that was used in pear characterization work (14), except in that the buffer pH was 8.3, as was used with apple tissue (13). Electrophoresis was performed on a multiple 16 cm (6.3 in) vertical slab electrophoresis cell (Model SE 700, Hoefer, San Francisco, California) using same conditions [5 watts per gel; gels thickness 1.5 mm (0.06 in)] as previously described (14); except that the cold room temperature was 3° C (37° F). The enzymic stains for peroxidase and acid phosphatase were done as described by Shaw and Prasad (17), and phenol oxidase following Hare (9); 3-amino-9-ethylcarbazole was used as a substrate for peroxidase, catechol for phenol oxidase and alpha-naphthyl acid phosphate for acid phosphatase. The *C. avellana* cultivar 'Barcelona' was used as a standard in each gel. A poorly defined region in the upper, low density, part of the gel stained lightly with all useful stains; this is attributed to enzyme polymers produced during extraction and was not useful for identification. Alkaline phosphatase, indoleacetic acid oxidase, alpha and beta esterases, glutamic oxaloacetic transaminase, leucine amino peptidase, and malic dehydrogenase were tested (17, 18) but these isozymic activities were: not found, poorly defined (esterase) or very dim (indole acetic acid oxidase). Isozyme patterns were visually identified and the gels were photographed with Positive Negative Film obtained from Polaroid Corporation, Cambridge, Massachusetts. Patterns were drawn after scanning the negative with a Hoefer GS 300 densitometer, using the output of the densitometer collected on a Model 3655 Yokagawa microprocessor equipped ('smart') recorder (Yokogawa Corporation of America, Shenandoah, Georgia). The recorder traces were normalized using the output of a specially built voltage-ramp device on the X-axis and the output of the densitometer on the Y-axis. Photographs used for figures were taken with a 35 mm camera using Tech Pan Kodak film (Eastman Kodak, Rochester, New York).

Probit analysis was used to determine values at zero input of a variable (5). This was done using a probit scale that 'linearizes' the normal distribution of a population. This permits the determination of data at zero input of a variable by linear extrapolation of data taken at known values of the variable. This method is commonly performed graphically (using 'probit' graph paper) (5, 15), although it can also be done mathematically (5). In this use the coordinates are: Y-axis linear, X-axis probit scale. The Y axis indicates the number of patterns repeated (1 = not repeated, 2 = repeated once, 3 = repeated twice, . . . , n = repeated n − 1 times). The X axis is [(number of patterns/sample size) × 100] and is plotted on a probit scale. This plot allows linear extrapolation to n = 0 which is the estimate of the percent patterns not yet found. For the purposes of comparisons between *Pyrus* and *Corylus* this analysis used the electrophoretic patterns from 114 diverse pear accessions including those already reported (14).

Results and Discussion

The photographs in Fig. 1 are representative of the acid phosphatase, peroxidase, and phenol oxidase isozyme patterns observed in this study. A diagramatic representation of all the isozymic patterns for the three enzymes studied and the numeric code assigned to each of these patterns is presented in Fig. 2. Table 1 gives the assignment of these isozymic patterns, utilizing this code, to the individual clonal accessions tested in this paper.

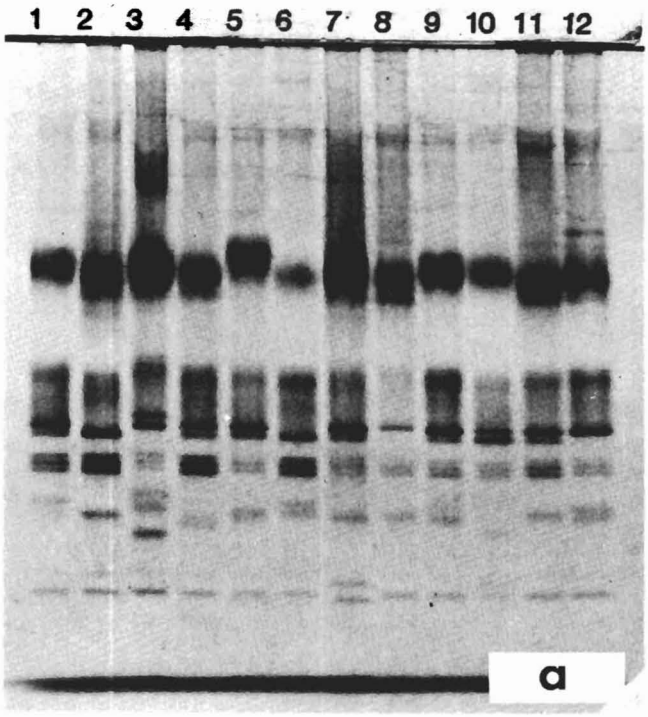
The patterns of isozyme separations suggest a rather wide variety of genotypes among the clonal accessions tested. To test reproducibility, each accession was extracted and submitted to electrophoretic-isozymic analysis a minimum of two times. All patterns assigned to each accession were reproducible. The isozymic patterns produced by the three enzyme staining systems from each accession tested remained constant throughout the sampling period, and had not changed at the date of this paper's galley proofs (February 20). In addition extracts from one-year, two-year and new-leaf-shoots in the same clone yielded the same isozyme patterns. Thus, the stability of isozyme patterns was similar to that reported in *Malus* (13) and *Pyrus* (14). However, the diversity of patterns was greater than that found in a total population of 114 diverse *Pyrus* accessions. Figure 3 shows the estimate, by probit analysis (5), of the total number of patterns in the NCGR *Corylus* and *Pyrus* collections. In these analyses the percent of patterns not yet found is estimated by the intercept of the zero line of the y-axis with the regression line generated by the number of repetitions of patterns as illustrated in a previous poster (15). For example, the lowest data point at the extreme left of Figure 3, an open circle, represents 35 *Pyrus* accessions which have the same common acid phosphatase pattern; while the three solid symbols at the upper right of the figure represent single occurrences of patterns among the population of *Corylus* accessions. In this estimate, large numbers of similar accessions are given little weight because they fall on the vertical segment of the biphasic curve (Fig. 3). Intercepts were calculated by regression formulae. The differences between the two genera for the intercepts of the pooled data using the common isozymes (acid phosphatase and peroxidase), were significant ($P < 0.01$). This suggests that the NCGR collection of the genus *Corylus* may be genetically more "isozymically" diverse than the collection of *Pyrus* accessions. Thus, this matter may be important to estimates of required numbers of accessions of these two genera in our germplasm collection.

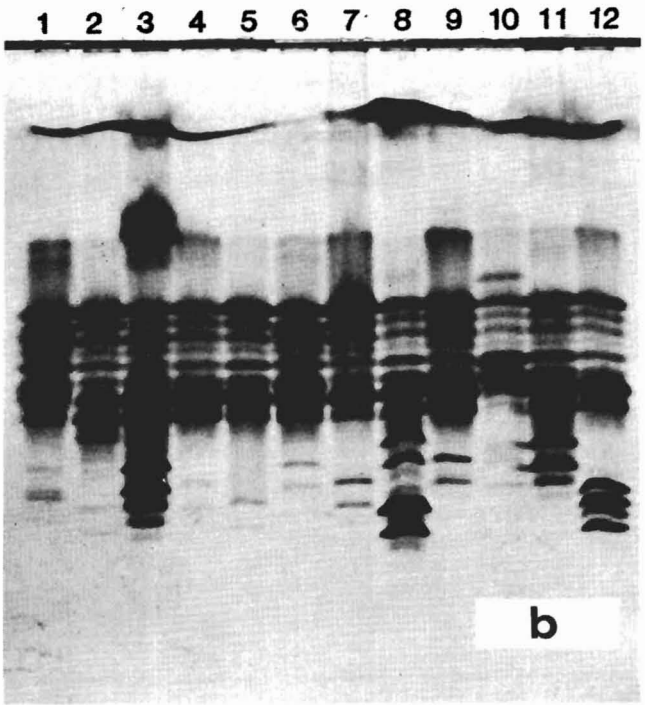
Acid phosphatase isozyme patterns from *Corylus* extracts showed relatively less diversity in the species and cultivars tested than those produced by phenol oxidase and peroxidase activities. This is similar to the situation in *Pyrus* (14, 15). All *Corylus* clonal accessions tested could be identified by their phenol oxidase and peroxidase patterns alone. However, acid phosphatase patterns are included, because of their potential usefulness in future work with *Corylus* germplasm.

The gradient polyacrylamide electrophoretic technique and subsequent isoenzymic staining appears to be useful for characterizing species and accessions of the genus *Corylus*.

Significance to the Nursery Industry

The procedures described for the identification of filbert (hazel) species and cultivars constitute an integral part of the program of conservation, characterization and study of National Clonal Germplasm Repository (NCGR), Corvallis, Oregon. The nursery industry, institutional breeding programs and independent breeders have NCGR germplasm collections at their disposal. This collection is a source of clonal plant material which contains many desirable characteristics including: disease resistance and cold hardiness.





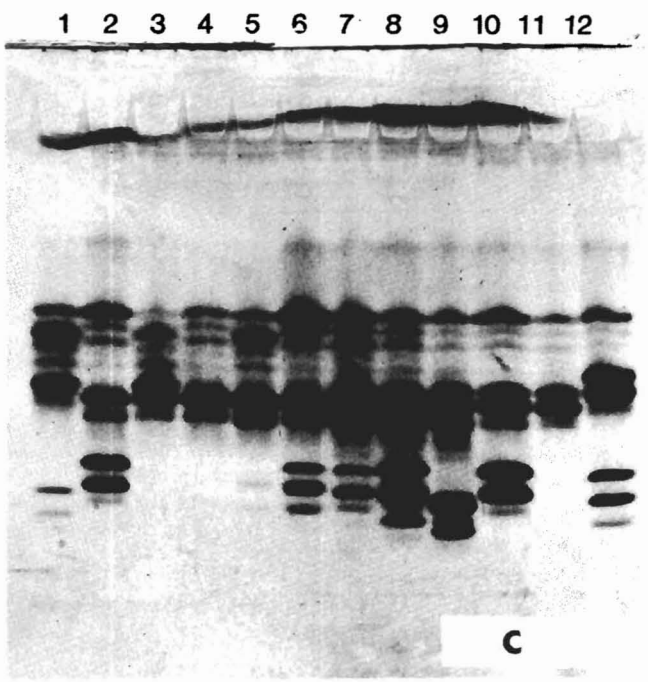


Fig. 1. **Photographs of polyacrylamide electrophoresis gels showing isozyme patterns of acid phosphatase, peroxidase and phenol oxidase. *Fig. 1a.* shows acid phosphatase staining patterns. The samples from left to right (1 to 12) represent extracts from: 1. *C. avellana* Barcelona standard (36), 2. *C. avellana* OSU 54-50 (93), 3. *C. sp.* (7), 4. *C. sp.* USOR 5-70 (125), 5. *C. heterophylla* (18), 6. *C. avellana* Gironenc (44), 7. *C. avellana* OSU 54-80 (97), 8. *C. sp.* BR-3-5 (134), 9. *C. sp.* USOR 6-73 (144), 10. *C. sp.* USOR 15-71 (165), 11. *C. sp.* BR-3-4 (133), 12. *C. sp.* BR-3-6 (135). *Fig. 1b.* Shows peroxidase staining patterns, samples numbered 1 through 12 are as in Fig. 1a. *Fig. 1c.* shows phenol oxidase staining patterns. The samples from left to right (1 to 12, NCGR number in parenthesis) represent extracts from: 1. *C. avellana* Barcelona standard (36), 2. *C. avellana* OSU 54-21 (85), 3. *C. maxima* Pellicule Rouge OSU (38), 4. *C. avellana* OSU 54-56 (94), 5. *C. avellana* OSU 54-39 (88), 6. *C. avellana* OSU 54-24 (87), 7. *C. avellana* Daviana (42), 8. *C. sp.* USOR 13-70 (141), 9. *C. sp.* BR-3-1 (131), 10. Trazel G-5 (169), 11. Chinese Trazel J-1 (170), 12. *C. sp.* USOR 13-71 (171).**

Table 1. Isozyme banding patterns for acid phosphatase, peroxidase and phenol oxidase found in 78 *Corylus* specimens, cultivars and clonal accessions. Examples of patterns can be found in Fig. 1 and diagrams of the patterns in Fig. 2. NCGR numbers (CCOR prefix omitted) are included for clone identification and to facilitate requests.

Species, cultivar or clonal accession (NCGR number)	Enzyme staining pattern		
	acid phosphatase	peroxidase	phenol oxidase
Corylus avellana Barcelona (36)	1	1	1
C. avellana Brixnut (26)	14	14	15
C. avellana Campanica (40)	1	31	28
C. avellana Casina (28)	2	24	23
C. avellana Cosford (41)	45	59	63
C. avellana Crossal de Constanti (75)	14	13	14
C. avellana Daviana (42)	33	51	49
C. avellana Ennis (11)	2	38	35
C. avellana Fitzgerald (27)	1	29	24
C. avellana var. *fusco-rubra* (39)	27	47	46
C. avellana Gassoway (54)	2	36	33
C. avellana Gem (23)	21	43	40
C. avellana Gironenc (44)	6	6	5
C. avellana Hall's Giant (16)	2	37	34
C. avellana Italian Red (30)	25	45	44
C. avellana Kruse (25)	26	32	45
C. avellana var. *heterophylla* (159)	1	30	4
C. avellana Montebello (17)	21	42	39
C. avellana Morell (6)	1	39	37
C. avellana Mortarella (51)	1	26	25
C. avellana Negret (8)	1	22	22
C. avellana Neue Riesennuss (10)	13	12	13
C. avellana Nonpareil (37)	22	41	38
C. avellana OSU 14-19 (35)	1	37	36
C. avellana OSU 14-84 (52)	1	25	24
C. avellana OSU 54-24 (87)	32	43	25
C. avellana OSU 54-21 (85)	29	49	48
C. avellana OSU 54-39 (88)	31	50	4
C. avellana OSU 54-50 (93)	2	2	2
C. avellana OSU 54-56 (94)	30	32	4
C. avellana OSU 54-60 (96)	15	15	16
C. avellana OSU 54-80 (97)	7	4	6
C. avellana OSU 54-81 (98)	24	45	42
C. avellana Pallaz (29)	1	35	31
C. avellana Ryan (3)	2	34	30
C. avellana Segorbe (20)	21	40	37
C. avellana Sivri Ghiaghli (32)	2	35	32
C. avellana Tombul (43)	23	46	43
C. avellana Tombul Ghiaghli (55)	20	26	27
C. avellana Tonda Bianca (21)	1	28	14
C. avellana Tonda di Giffoni (22)	23	44	41
C. avellana Tonda Gentile Romana (5)	1	33	29
C. avellana Woodford (12)	2	25	24
C. colurna var. *chinensis* (19)	4	23	20
C. colurna var. *chinensis*[z] Chinese Trazel J-1 (170)	37	32	16
C. colurna var. *chinensis*[z] Chinese Trazel G-4 (174)	43	43	61
C. colurna var. *lacera* (34)	16	16	17
C. colurna Turkish Trazel[z] G-5 (169)	36	49	52
C. heterophylla (18)	5	5	4
C. heterophylla A (146)	42	43	not detected
C. maxima Pellicule Rouge OSU (38)	29	32	16
C. sp. Riccia di Talanico (45)	1	27	26
C. sp. BR-3-1 (131)	35	53	51
C. sp. BR-3-3 (132)	1	55	57
C. sp. BR-3-4 (133)	10	9	10
C. sp. BR-3-5 (134)	8	7	7
C. sp. BR-3-6 (135)	11	10	11
C. sp. BR-4-1 (136)	14	17	18
C. sp. Eastoka (148)	28	48	47
C. sp. Chinese[z] Trazel G-6 (138)	40	43	55
C. sp. Estrella No. 1 (139)	42	57	59
C. sp. Estrella No. 2 (140)	39	54	54
C. sp. Moturk-D (137)	41	56	58
C. sp. USOR 2-67 (161)	16	18	19
C. sp. USOR 1-67 (162)	17	19	20
C. sp. USOR 5-70 (125)	4	4	4
C. sp. USOR 13-70 (141)	34	52	50
C. sp. USOR 5-71 (166)	9	8	9
C. sp. USOR 13-71 (171)	38	52	53
C. sp. USOR 20-71 (163)	18	20	20
C. sp. USOR 1-72 (142)	1	43	60
C. sp. USOR 3-72 (177)	44	58	62
C. sp. USOR 1-73 (143)	12	11	12
C. sp. USOR 3-73 (160)	19	21	21
C. sp. USOR 6-73 (144)	5	6	8
C. sp. USOR 8-73 (145)	22	43	56
C. sp. unidentified (7)	3	3	3
C. vilmorinii (14)	1	32	24

[z]Trazels are the product of crosses between species in which *C. avellana* is one parent, the other is indicated in table (M.M. Thompson, personal communication).

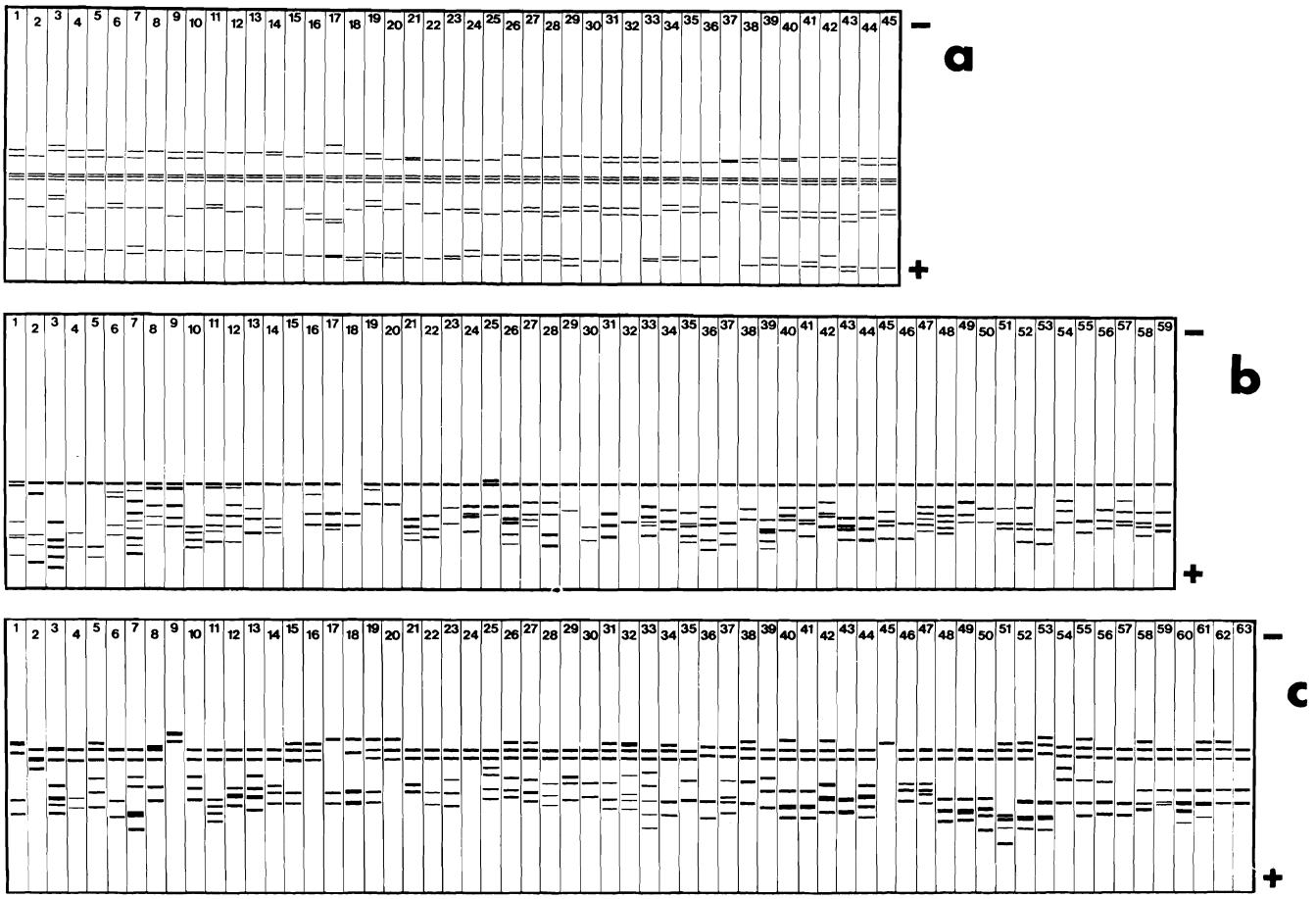


Fig. 2. Diagramatic representation of isozyme patterns for 78 *Corylus* species and accessions. The pattern is indicated by adjacent numbers. *Figure 2a* shows acid phosphatase patterns; *Fig. 2b* peroxidase patterns; *Fig. 2c* phenol oxidase patterns. The correspondence between pattern numbers and species or cultivars can be obtained from Table 1.

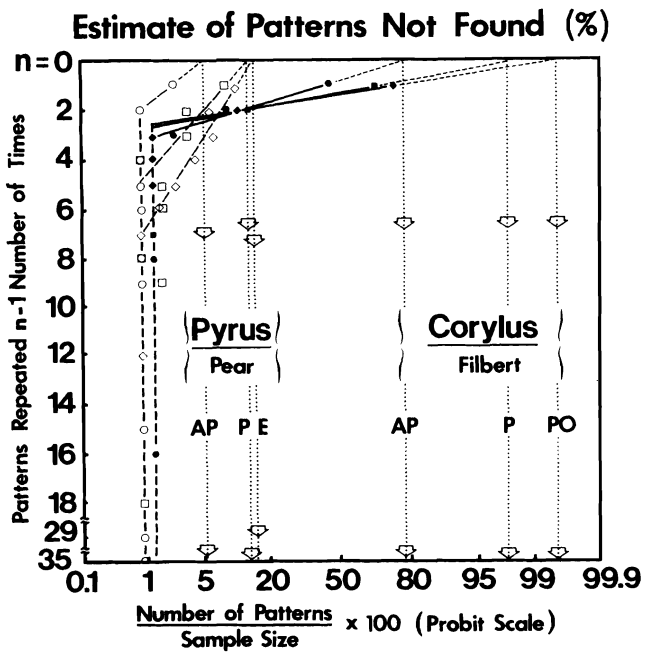


Fig. 3. Estimation of number of patterns by probit analysis. Y-axis: n=1, pattern not repeated; n=2, pattern repeated once; n=3, pattern repeated twice. X-axis: number of patterns with the same n number divided by sample size (total number of plant accessions tested). Intercept of extension of data lines with the upper (zero) horizontal axis yields the estimate of percent patterns not yet found. Continuous lines *Corylus* data, discontinuous lines *Pyrus* data. Acid phosphatase patterns: *Corylus* closed circles, *Pyrus* open circles; esterase: *Pyrus* only open rhomboids (diamonds); peroxidase: *Corylus* closed square, *Pyrus* open squares; phenol oxidase patterns: *Corylus* only closed rhomboids (diamonds).

This paper describes a dependable, simple procedure for identification that can be used regardless of plant age. This method will help prevent the costly consequences of mislabeling of clones.

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