Characterization of Quince (*Cydonia*) Cultivars Using Polyacrylamide Gel Electrophoresis[1]

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Abstract

An investigation of available *Cydonia* (*Cydonia oblonga* Mill., quince, membrillo) germplasm by isozyme staining of anionic polyacrylamide gradient electrophoresis gels is described. The isozymes of acid phosphatase, esterase, peroxidase and phenol oxidase showed most diversity and usefulness for this purpose. Eleven groups of quince and two groups of *x Pyronia* (quince-pear crosses) were distinguished by their isozyme patterns. These patterns distinguish between groups of clonal accessions, and the patterns were constant for each accession during the test period (December, 1986 to August, 1987). Thus, these patterns were considered to represent genetic characteristics suitable for identification purposes. The diversity of isozyme patterns was much less than in *Corylus* and *Pyrus* populations previously sampled; and less than that of a restricted pool of apple cultivars previously examined.

Index words: acid phosphatase, *Cydonia*, electrophoresis, esterase, isozyme diversity, membrillo, peroxidase, plant fingerprinting, phenol oxidase, *x Pyronia*, quince

Introduction

Chemical identification (fingerprinting) of plant species and cultivars has received increased attention (1, 3, 4, 5, 6, 8, 11, 13, 18, 20, 21, 23, 24, 30, 31) 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. The National Clonal Germplasm Repository (NCGR) system, collects, maintains, identifies and characterizes clones of selected crop genera (16). The NCGR at Corvallis, Oregon is responsible for eight major genera including a world collection of *Pyrus* (pears) (28, 29) which comprises about 1,800 accessions. Together with the pears, a small collection of *Cydonia oblonga* Mill. (membrillo, quince) is maintained.

Cydonia has been used for centuries as a dwarfing rootstock for pear (15, 19, 26), and its fruit is sold frequently in specialized markets on the west coast of the U.S. A chemical identification procedure previously used to identify *Malus* (apple), *Pyrus* (pear) and *Corylus* (filbert, hazel) species, cultivars and clonal acessions, was tried with *Cydonia* (membrillo, quince) cultivars and clonal accessions. The objective was to investigate by isozyme staining of anionic polyacrylamide gradient electrophoresis gels the restricted germplasm pool of this genus. Limited germplasm is associated with poor crop adaptability (19) and believed to be a consequence of limited natural range (27) and self-pollinizing and self-fruiting habits (12). In addition, because of wars in its native range (which includes Iran and Afganistan) it is difficult to collect wild *Cydonia* accessions.

The genus *Cydonia* presents an *in vivo* model of what might happen to a major crop genus, such as *Pyrus*, were its germplasm pool restricted by loss of wild species and less common cultivars. Available quince accessions lack genetic resistance to many diseases and do not tolerate wet soils or cold winters (19).

In previous investigations of other genera [*Corylus* (1), *Malus* (20, 23), *Pyrus* (8, 21, 22)] isozyme patterns that identified each clone remained constant throughout the year, and were not affected by the age of the plant from which the shoots were collected. This is attributed to the diversity of tissues found in stems, which not only include phloem, xylem and cambium, but also photosynthetic, active tissues (7). Thus, specific isozymes for all these tissues (17) are present. Since these stem isozyme patterns remain constant this method can be used with: very young specimens before phenotypic morphological characteristics such as those of flower or fruit appear, mature trees when these transitory characteristics are not available, or budwood from nurseries or quarantined sites that are remote from the laboratory as some accessions described in this paper.

Materials and Methods

Plant material was obtained from the NCGR collection, Corvallis, Oregon, commercial nurseries or quarantine sites

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as in acknowledgement section. Quarantine and nursery material was supplied as budwood. Quarantine material was destroyed after use as required. One year old shoots were collected from December 1986 through August 1987. The protein extraction technique used for *Cydonia* was the same as that used in pear characterization (21). Stems were stored at −30°C (−22°F) before extraction. This treatment is known to induce polyphenol oxidase activity (7). Electrophoresis was performed on a multiple 16 cm (6.3 in) vertical slab electrophoresis cell (Model SE 700, Hoefer, San Francisco, California) using similar conditions [20 miliamperes with a 7.5 watts per gel upper limit; gel thickness 1.5 mm (0.06 in)] as previously described (21); except that the cold room temperature was 3°C (37°F). The enzymic stains for peroxidase, esterase and acid phosphatase were done as described by Shaw and Prasad (25), and phenol oxidase following Hare (14). Enzyme substrates were: beta-naphthyl acid phosphate for acid phosphatase; alpha-naphthyl acetate for esterase; 3-amino-9-ethylcarbazole for peroxidase; and catechol:p-phenylenediamine (1.6:0.8 g/l) for phenol oxidase. The *Cydonia* cultivar 'East Malling Quince type A', an Angers quince, 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. Alcohol dehydrogenase, alkaline phosphatase, indoleacetic acid oxidase, beta esterase, glutamic oxaloacetic transaminase, leucine amino peptidase, and malic dehydrogenase were tested (25, 27), but did not yield satisfactory patterns. Isozyme patterns were visually identified and the gels were photographed with Positive-Negative Film (Type 55, Polaroid Corporation, Cambridge, Massachusetts). One photograph for the figures was taken with a 35 mm camera using Tech Pan Kodak film (Eastmand Kodak, Rochester, New York). The other photographs were professionally reproduced from Polaroid negatives. Development of figures was done in such a way to selectively enhance those regions of the gels that were used for analysis.

Probit analysis is used to determine values at zero input of a variable (1, 10, 22). This is 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) (1, 22), although it can also be done mathematically (10). In this use the coordinates are: Y-axis linear, X-axis probit scale. The Y axis indicates the number of patterns repeated (0 = not found, 1 = not repeated, 2 = repeated once, 3 = repeated twice, ---, n = repeated n − 1 times). The X axis is number of patterns × 100/sample size 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.

Results and Discussion

The photographs in Fig. 1 are representative of the acid phosphatase (1d), esterase (1c), peroxidase (1a), and phenol oxidase (1b) isozyme patterns observed in this study. A diagramatic representation of all the isozymic patterns for the four 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.

Fig. 2b, phenol oxidase isozyme patterns shows a region of undefined band structure. This area may contain additional information but was not considered useful for the purposes of this paper. Quince preparations yield gels with slightly stronger background, yielding slightly less distinct photographs, than those of pear (8, 21) or apple (23); and pear extracts yield slightly less distinct photographs than those of *Corylus* (1). This is attributed to a need to tailor the trailing ion in the upper buffer (now glycine) by substituting a mix of amino acids with variable pKa's. This effect did not reduce the usefulness of the method. In Fig. 2c, pattern 1a represents heavily-overloaded esterase-stained gels of extracts, which when normally loaded yield esterase pattern 1. This situation may arise from low molecular weight components of the extract acting as trailing ions and thus promoting better resolution. Acid phosphatase isozyme patterns showed relatively less diversity in the species and cultivars tested than those produced by esterase, phenol oxidase and peroxidase activities. This is similar to the situation in *Pyrus* (21, 22), *Malus* (20, 23) and *Corylus* (1). Acid phosphatase patterns may represent parts of metabolite transport mechanisms (e.g. 7) and a search for additional *Cydonia* clones with different acid phosphatases may provide clues to the lack of adaptation of quince rootstocks to wet soils (19).

All patterns assigned to each accession were reproducible. Since isozymic patterns produced by the four enzyme staining systems from each accession tested remained constant throughout the sampling period, these patterns were considered to represent genetic characteristics suitable for identification purposes. Thus, the stability of isozyme patterns was similar to that reported in *Corylus* (1), *Malus* (20, 23), and *Pyrus* (21, 22). Eleven groups of *Cydonia* were distinguished (Table 1). *x Pyronia* (quince × pear crosses) yielded two additional groups. The low number of isozyme patterns obtained suggest a restricted number of genotypes among the clonal accessions tested. Quince rootstocks are frequently reproduced by seed from selfed-parent trees (19); therefore, different isozyme patterns of similarily named quince rootstock are more probably due to genetic segregation during selfing of parent trees, rather than errors in labeling.

Figure 3 utilizes data for *Cydonia* and that of a pool of cultivars of *Malus* (taken from Table 2 of reference 23) to develop estimates of clonal diversity by probit plot methods (1, 10, 22). What the probit plot does in this application is give a number and a rationale for what is intuitively perceived: 'if a small number of patterns is found in a sample of a population, then the number of total patterns in that population is also small' (P. Breen, pers. communic. 1987). The restricted pool of *Malus* (23) was selected because it was entirely composed of cultivars and thus more like the pool of *Cydonia* than the pool of accessions examined in other genera. For comparison, data on *Pyrus* and *Corylus* is also plotted in the lower part of the figure.

Fig. 3 shows estimates, by probit analysis (10), of the total number of patterns not (yet) found for these four genera. Probit analysis linearizes a normal curve (or peak) yielding a plot that has the appearance of an inverted V. In this use only one half of the inverted "V", a line sloping upward from left to right is shown. Linearization occurs because

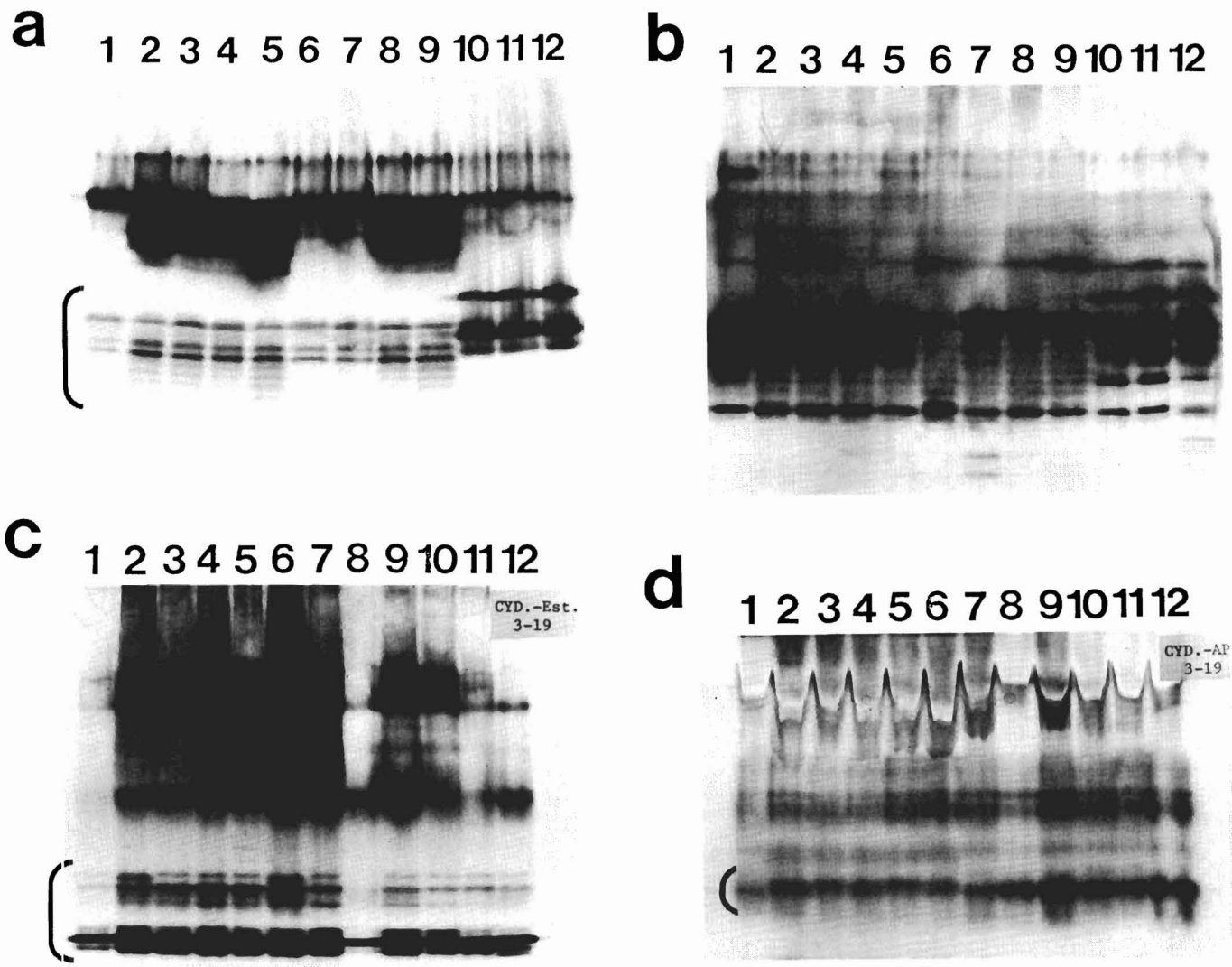

Fig. 1. **Photographs of polyacrylamide electrophoresis gels showing isozyme patterns of esterase, peroxidase, phenol oxidase and acid phosphatase. For notations for source of samples see Table 1. Fig. 1a. Shows peroxidase staining patterns (photographed with 35mm camera). The samples from left to right represent extracts from: 1. Quince A standard, 2. W2/494 B (old block), 3. Fontaney 498 A (old block), 4. WF/17/495 A (old block), 5. Pillnitz 4 (old block), 6. Quince C (nursery), 7. Quince BA 29 (nursery), 8. Quince C (old block), 9. CAUC/1/154 (old block), 10. *x Pyronia veichii* (NCGR), 11. *x Pyronia* Pyn 2 (NCGR), 12. *x Pyronia* Pyn 1 (NCGR). Fig. 1b phenol oxidase staining patterns. Samples are as in Fig. 1a. Fig. 1c. shows esterase staining patterns. Brackets indicate area used for identification. The samples from left to right (1 to 12) represent extracts from: 1. Quince A (standard), 2. Sekergevek, 3. Tekes, 4. Limon, 5. Havron, 6. Emek, 7. Quince C (nursery), 8. Quince A (sucker, student pear block Lewis Brown farm), 9. Quince C (sucker, student pear block, Lewis Brown farm), 10. Quince BA 29 (nursery), 11. Quince C (old block), 12. Pineapple (Lisle). Fig. 1d Acid phosphatase staining patterns, samples as in Fig. 1c.**

the x-axis (horizontal axis) coordinates of the data points are distributed following a probit scale (see Fig. 3). Probit plots are used to resolve curves with more than one component, because such plots yield distinct linear segments. When this occurs, the ''legs'' of the inverted V are not straight, but are composed of distinct linear segments (see Fig. 3). In this method if a large numbers of accessions with the same patterns are used, this causes little effect because they fall on the vertical segment of the biphasic curve (lower left Fig. 3).

Since these curves are linearized, intercepts can be determined by linear extrapolation of the regression line. In these probit analyses the percent of patterns not yet found can be estimated (1, 22) thus: (a) calculate and trace the regression line of the data for each number of repetitions of patterns. (b) Using this regression line find its intercept with the horizontal extension at $n = 0$ on y-axis. At $n = 0$ on the y-axis, the number of times a pattern has been found, is zero. Thus this intercept is the ''zero point''. (c) From the zero point a vertical line (in Fig. 3 a dotted line down) to the probit scale of the x-axis yields an estimate of % of patterns not yet found. An example is given below.

In the lower part of Fig. 3 the linearized curve indicated by the open rhomboids (diamonds) represents the data for *Pyrus* esterase patterns. In this linearized curve ignore the vertical segment at higher values of n ($n > 7$ on y axis), and observe the linear segment at $n < 7$ (upper left of lower part of Fig. 3). In this part of the linearized curve the open diamond symbols form a straight line with a slope of about 55 degrees to the horizontal. Extrapolation of regression line of this data yields an intercept with the horizontal extension of $y = 0$. This intercept has coordinates $y = 0$ and

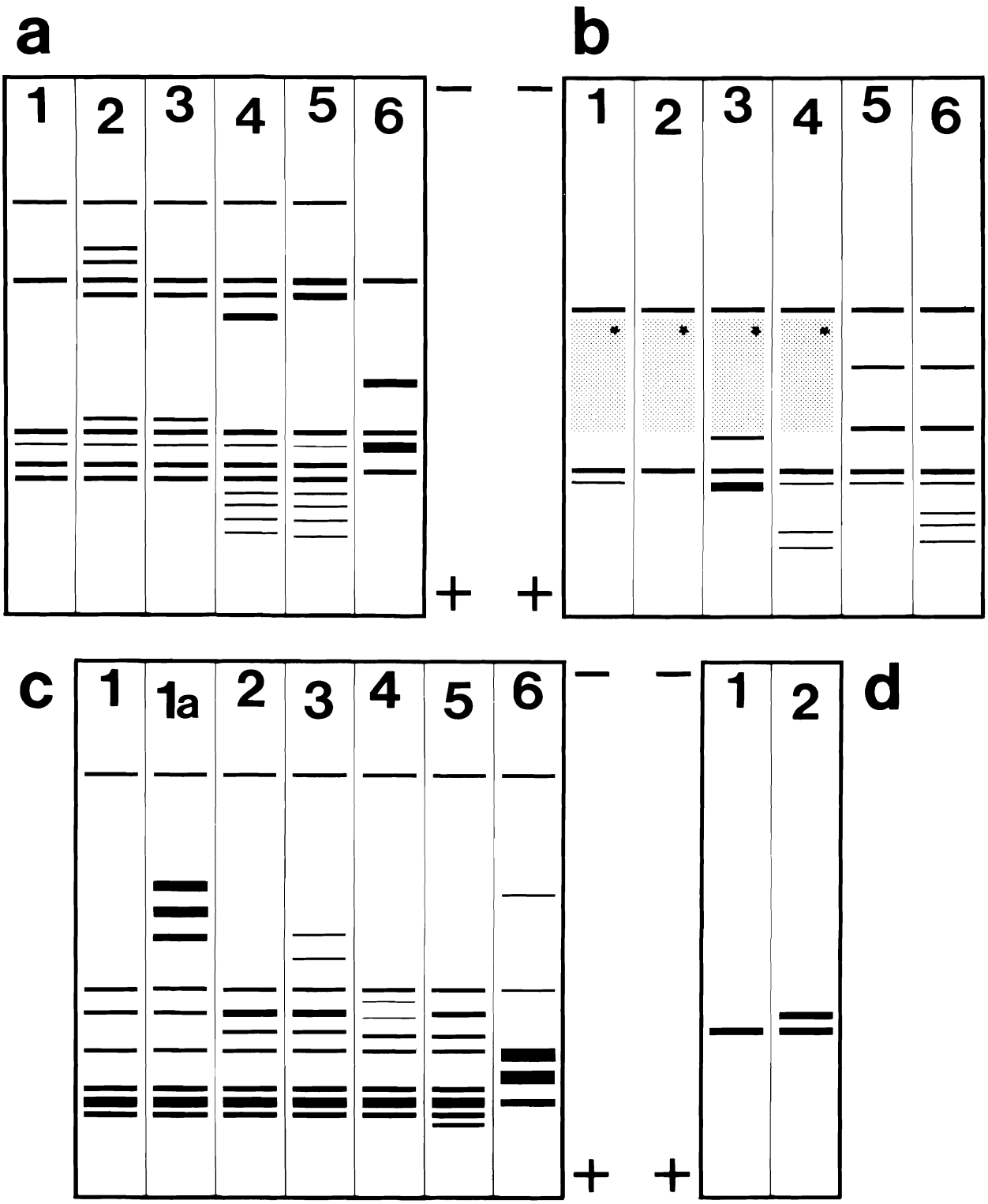

Fig. 2. Diagramatic representation of isozyme patterns for *Cydonia* accessions. The pattern code is indicated by adjacent numbers. Fig. 2a shows peroxidase patterns. Fig. 2b phenol oxidase patterns, shaded area (see asterix) shows region of undefined band structures. Fig. 2c esterase patterns Lane 1a indicates presence of polymeric forms when gel is overloaded. Fig. 2d acid phosphatase patterns. The correspondence between pattern numbers and species or cultivars can be obtained from Table 1.

Table 1. **Isozyme banding patterns for acid phosphatase, esterase, peroxidase and phenol oxidase found in *Cydonia* cultivars and clonal accessions. Examples of patterns can be found in Fig. 1 and diagrams of the patterns in Fig. 2. Notations include: old block, original Westwood collection at NCGR site; Pyn, *x Pyronia* notation on NCGR records; nursery and student (pear) block, Oregon State University, Horticulture Dept. collections, at Lewis Brown Farm; Lisle, Lisle collection; GD, USDA Plant Introduction Station quarantine collection at Glenn Dale; sucker, suckers from rootstock in student pear block, Lewis Brown Farm, Oregon State University. Fontenay 498 A and Pillnitz 4 extracts only yield phenol oxidase pattern 3 (3*) when overloaded, when the gels are not overloaded with these extracts pattern 1 is displayed. However, pattern 3 is not obtained by overloading extracts of other clones which normally yield pattern 1.**

	Enzyme staining pattern			
Cultivar or Clonal Accession	**Peroxidase**	**Esterase**	**Acid Phosphatase**	**Phenol Oxidase**
Quince A (old block)	1	1	1	1
Quince C (nursery)	1	1	1	1
A (Sucker, student block)	1	1	1	1
C (sucker, student block)	1	1	1	1
Orange (Lisle)	1	1	1	1
Cooke's Jumbo (Lisle)	1	1	1	1
Champion (Lisle)	1	1	1	1
Van Deman (Lisle)	1	4	1	1
Quince BA 29 (nursery)	1	1	1	4
Apple (Lisle)	1	5	1	1
Pineapple (Lisle)	1	5	1	2
Q 25982 Sekergevek (GD)	2	2	2	1
Q 25979 Ekmek (GD)	2	2	2	1
Q 25981 Tekes (GD)	3	2	2	1
Q 25980 Limon (GD)	3	2	2	1
Q 25978 Havron (GD)	3	3	2	1
Pillnitz 4 (old block)	4	1	1	3*
Quince C (old block)	5	1	1	1
Quince W2/494 B (old block)	5	1	1	1
Quince WF/17/495 A (old block)	5	1	1	1
CAUC/1/154 (old block)	5	1	1	1
Fontenay 498 A (old block)	5	1	1	3*
x Pyronia veitchii	6	6	1	5
x Pyronia (Pyn 2)	6	6	1	5
x Pyronia (Pyn 1)	6	6	1	6

x = approximately 18% (as indicated by the vertical dotted line marked by descending arrows and the letter E for esterase). Thus, 18% is the approximate percent of patterns of *Pyrus* esterase yet to be found. The data for *Pyrus* peroxidase (open squares, P) yields similar results. However, examining the data for *Corylus* (lower part of Fig. 3) it is clear that here a much higher numbers of patterns are yet to be found. This data has been examined statistically (1) and as this figure indicates there are more isozyme patterns for these enzymes in *Corylus* than in *Pyrus*.

Comparing *Malus* (upper part of Fig. 3) with *Pyrus* the estimate of % patterns not found is larger for *Malus*. This is consistent with the larger sample size of our *Pyrus* investigations (21). However, comparing approximately equal sample sizes of *Cydonia* (upper part of Fig. 3) and *Malus*, estimates of patterns not found are still greater for *Malus*.

Since proteins are direct gene products and their electrophoretic behaviour (isozyme patterns) is a direct consequence of their genetically determined aminoacid composition and sequence; a greater number of patterns indicates greater genetic diversity of the enzymes examined. Thus, of the four genera investigated so far *Cydonia* has less enzymic diversity and thus is, probably, the least genetically diverse genera. The differences between estimates of patterns not found for isozymes common to both *Malus* and *Cydonia* were significant ($P < 0.05$). This suggests that the cultivars of *Cydonia* investigated here represent a gene pool even less genetically diverse than that of a restricted pool of apple cultivars; and considerably less than that found in *Pyrus* and *Corylus* collections previously examined (1) Fig. 3b. Thus, it is important to collect as many diverse *Cydonia* clones as are available to maintain germplasm diversity for future breeding purposes. Since the numbers of available diverse *Cydonia* clones is small this is not expected to greatly increase NCGR costs, and since *Cydonia* can be crossed with *Pyrus* this increases the effective germplasm pool for both genera.

These data support the concept that given the restricted range of the genus *Cydonia*, a correspondingly restricted germplasm pool may be expected. Two responses to this situation are suggested: all available quince germplasm be collected and screened for diversity and attention be given to producing more *x Pyronia* (quince-pear crosses) to facilitate the introduction of the more diverse *Pyrus* germplasm into quince accessions.

Significance to the Nursery Industry

The procedures described for the identification of quince (membrillo) cultivars constitute an integral part of the program of conservation, characterization and study at the National Clonal Germplasm Repository (NCGR), Corvallis, Oregon. The nursery industry, institutional breeding programs and independent breeders have NCGR germplasm collections at their disposal. This particular investigation concentrates its attention on quince, a dwarfing root stock

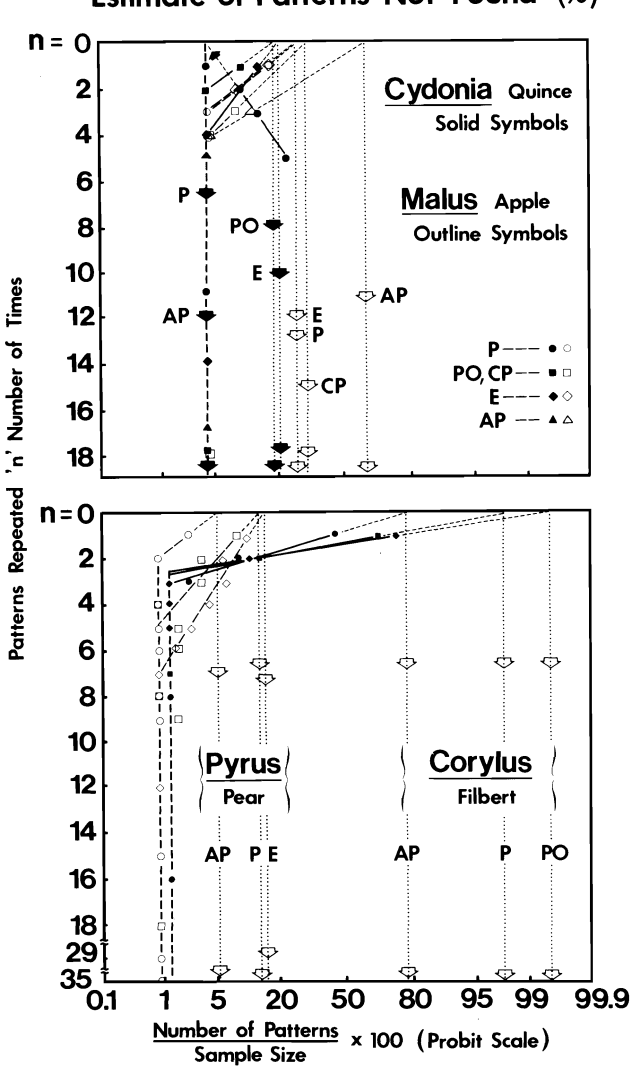

Fig. 3. **Estimation of number of patterns not yet found by probit analysis. Sources of data. Fig. 3a *Cydonia*, excluding *x Pyronia*, 22 accessions, from Table 1; *Malus*, 22 cultivars [Spartan and Wijick consider as distinct cultivars, sports considered as same cultivar isozymically (8, 23)], data from Table 2, of reference (23). Fig. 3b taken from reference (1), 114 *Pyrus* accessions, 78 *Corylus* accessions. 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. These intercepts are indicated with vertical discontinuous lines. Upper case letters on these lines indicate intercept of: AP, acid phosphatase; CP, cationic peroxidase; E, esterase; P, peroxidase and PO, phenol oxidase. Fig. 3a *Cydonia* data closed symbols: acid phosphatase patterns, closed triangles; esterase, closed rhomboids (diamonds); peroxidase, closed circles; phenol oxidase patterns, closed squares. *Malus* isozyme patterns are indicated by open symbols which correspond to the same enzymes as the corresponding closed symbols for *Cydonia* isozymes, except that cationic peroxidase (open squares), replaces phenol oxidase. Fig. 3b 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).**

for pear. This paper describes a dependable, simple procedure for identification that can be used on shipped quince budwood. This method will help in the search for sources of quince germplasm that may include clones that are more resistant to wet soil conditions and diseases that detract from the usefulness of quince as a dwarfing root stock for pear and as a fruit crop.

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Influence of Nitrogen and Phosphorus on Growth and Tissue N and P Concentration in *Salvia greggii*[1]

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Abstract

Studies were conducted at the Texas Agricultural Experiment Station, Dallas to determine nitrogen and phosphorus requirements of *Salvia greggii* Gray. (autumn sage), a resource-efficient landscape plant for the Southwest. Maximum growth of potted rooted cuttings used in the studies was obtained with application of 200 mg/liter (ppm) N and 50 mg/liter (ppm) P, although fertilization with 150 mg/liter (ppm) N and 30 mg/liter (ppm) P would produce near maximum growth. Tissue levels should be > 2.2% N and > 0.20% P for these elements not to limit growth.

Index words: fertilizer, landscape plants, native plants, plant analysis, autumn sage

Introduction

Resource efficient native landscape plants are gaining in popularity, particularly in the Southwest, where water is frequently limited or rationed. Most native plants with landscape potential are taken from locations such as the Chihuahuan Desert where annual rainfall is 25–30 cm/yr (10–12 in/yr) (7) and soil fertility is low. Little information is available regarding nutritional requirements for these plants under growth conditions required in containerized nursery production. Nursery production is an essential step in providing native plant material for landscape use. Nitrogen fertilizer requirements for nursery production of *Leucophyllum candidum* (3), *Arbutus xalapensis* (4), and N effects on rooting by *Leucophyllum* (6) have been determined.

Salvia greggii (autumn sage) is an attractive, hardy native perennial shrub that blooms much of the summer and is an excellent landscape plant for the Southwest. Since there is no information relative to containerized production of this plant, studies were conducted at the Texas Agricultural Experiment Station at Dallas to determine nitrogen (N) and phosphorus (P) fertilizer requirements and critical tissue N and P levels.

Materials and Methods

Rooted tip cuttings of *S. greggii*, approximately 6 cm (2.4 in) in length, were placed in 15 cm (1 gal) plastic pots containing 2 parts perlite:1 part vermiculite (by vol). Nitrogen treatments were applied by irrigating weekly with water containing variable levels of N and biweekly with water containing three different levels of P. Nitrogen levels were 12.5, 25, 50, 100 and 200 mg/liter (ppm) and P application rates were 0, 25, and 50 mg/liter (ppm). Water extracts (saturated paste) of medium from the zero P treatments contained 0.3 mg P/liter; thus the zero P treatment was actually 0.3 mg P/liter and each treatment level was elevated by that amount. The source of N was NH_4NO_3 and the P source was H_3PO_4. All pots were irrigated every 2 weeks with nutrient solution lacking N and P as described by Hoagland and Arnon (5) except chelated iron was substituted for iron tartrate. Media pH was approximately 6.3. Sufficient fertilizer solution was applied to provide about

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