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# Plant Regeneration, Somaclonal Variation and Resistance to Late-blight in Tomato

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## Abstract

Genotypes were quantitatively different in shoot and rot-forming capacity from cotyledon and hypocotyl explants. Tomato genotypes LA 1623, LA1456, LA1546 and FMX-93 had a higher frequency of shoot regeneration and root formation the Nova and Rockingham. Shoot formation by cotyledon explants was found to be superior to shoot formation by hypocotyl explants in all genotypes except LA 1546 in which the hypocotyl was superior in producing shoots. Somaclonal variation with altered leaf shape, plant height and seedling viability were noted in the $R_1$ generation, with increased and decrease resistance to late-blight in mutants.

## Introduction

A repeating theme in plant tissue culture is the genotypic variation in culture response. Conditions optimal for plant regeneration in one cultivar tail to produce plants in another cultivar of the same species. This has been a nagging problem in plant tissue culture. According to Welander and Maheswaran (1992), novel method of crop improvement involving gene transformation, protoplast fusion, somaclonal variation, and mutation breeding depend on tissue culture for regeneration of new plants. Success with these approaches requires, in part, the development of an efficient plant regeneration system. For gene tagging with transposable elements, one of the conditions to be satisfied is that the recipient plant must be amenable to regeneration. For tomato, differences between cultivars and mutants in shoot and callus induction on primary explants have been described by many authors. The main sources of variability for the breeder have been mutation and crosses with other cultivars, wild lines or other species followed by selection for the desired characters. Regeneration from *In vitro* culture has disclosed another source of exploitable variation, generally referred to as somaclonal variation (Larkin and Scowcroft, 1981). Somaclonal variation is appealing to the breeder because it often occurs at a higher frequency than chemically induced rnutagenesis. For instance, 72 percent variants have been reported in rice regenerants (Sun *et al*., 1983). There is evidence that increased resistance to fungal pathogens can be generated by somaclonal variation (Shepard *et al*., 1980). The most difficult obstacle in identifying the optimum *In vitro* treatment is that the occurrence of lines with improved traits is a rare event, the majority of variants produced being inferior in some respect to the parent. Nevertheless, some tissue culture derived cultivars, have been released (Duncan, 1997). Since resistance against tomato late-blight race 1 is not available, increased blight-resistance in tomato might be obtainable by this mean. The objectives in these investigations were (a) to evaluate the relative *In vitro* morphogenic responses of hypocotyl and cotyledon explants of six tomato genotypes with some specific resistance to late-blight and of one genot) susceptible to late-blight (b) Since variation in sensitivity *P. infestans* has not been investigated, the second object of this study was to screen R1 progeny from somaclonal for changes in sensitivity to this pathogen.

## Materials and Methods

These studies were conducted at School of Biology Sciences, University College of North Wales Bangor, UK.

### Regeneration

**Plant Material:** The six tomato genotypes were chosen because they had either resistant or susceptible reactions *P. infestans* tomato race 0. In experiment I, FMX- (susceptible), Nova (resistant), LA 1456 (resistant), 1546 (resistant), LA 1623 (resistant), and in experiment Rockingham (resistant) was included in addition accessions in experiment I.

**Germination of Seeds:** In both the experiments tomato seeds were planted in soil-less compost (Levington IU Fisons, Ipswich, U.K.) in seed trays. Sown seeds were covered with a thin layer of compost and were water gently. Trays with seeds were placed in a growth chamber at 25 ± 1°C with 70-80 per cent relative humidity and h day length. Subsequently, trays were watered when decay.

**Preparation of Cotyledonary and Hypocotyl Segments:** The experiments 1 and 11, 13 and 10 day-old seedlings was expanding first leaves were utilized as explant sourd, respectively. All plant material was surface- sterilized immersion in 8.0 percent (v/v). domestos bleach solution (Lever Bros Ltd, London, U.K.) for 30 min, followed by food washes with sterile tap water. Two cotyledonary segment (5 mm in length) each including part of the central were excised from each cotyledon. Two hypocotyl segments 5 mm in length were obtained immediately beneath the cotyledons. These segments were the randomly planted onto culture medium.

**Culture of Explants:** The nutrient medium used (MS medium) in both experiments consisted of the inorganic salts and vitamins as described by Murashige and Skoog (1962) supplemented with sucrose 2 percent (w/v), 0.8 percent (w/v) agar, 2 mg /1 zeatin and 0.1 mg/1 indole acetic acid. The PH of the medium was adjusted to 5.8 before the addition of agar. All media were autoclaved for 15 minutes at 121°C except for growth regulator stocks, which were filter-sterilized and added to the medium after autoclaving in the appropriate concentrations. Four explants were placed on media in each 90 mm diameter pre-sterilized, disposable, plastic Petri dish (Fisher, Inc) and sealed with catering film. In each treatment there were four dishes. The dishes were incubated in a growth chamber at 25 ± 1°C with 70-80 percent relative humidity and 16 h day length. Explants were transferred to fresh medium after four weeks. After 8 weeks shoots produced by explants were excised and transferred singly to rooting medium, based on the MS formulation but with 1.0 percent (w/v) sucrose and 0.6 percent (w/v) agar, pH 5.8 in plastic vessels (60 mm x 60 mm x 60 mm) (Magenta boxes) (Sigma) and incubated as above. Nine shoots were cultured in each Magenta vessel.

**Production of Plants:** The rooted shoots of experiment I were potted singly in soil-less compost (Levington M3) in small pots (four cm diameter) and covered with plastic film. The potted plants were placed on a capillary mat and maintained in the glasshouse at a day temperature of 18±3°C and night temperature of 12 ± 2°C with supplementary illumination at Penn-y-ffrid Field Station. Holes were poked in the plastic film after three days. The covering was completely removed about one week later. After two months plants were transferred into pots 20 cm diameter in the same compost and placed in the cold house at a day temperature of approximately 16°C arid night temperature of 12°C. A Chempak liquid fertilizer (No. 4) of a balanced formula (NPK 15-15-30) was given weekly to the plants at the recommended concentration. Each plant received about 1.5 litre of fertilized water . The side shoots of each plant were removed soon after they appeared.

**Data Analysis:** The data were transformed by a $\sqrt{X} + 1/2$ transformation prior to analysis since some of the values were less than 10 and especially zero were present and then the analysis of variance technique and LSD test at the 5 per cent level of probability was employed to compare the significance among means (Steel and Torrie, 1980).

**Somacional Variation**

**Plant Material:** The inflorescence of rooted shoots of experiment 1 were covered with Stainshury's Baton Bags 150 x 390 mm (W. R. Grace Ltd. st. Neots, Carnbs, England). The seed was extracted from 200 tissue culture -derived lines and is available for studies of somaclonal variation.

The 48 R1 families of the most resistant accession LA 1623 to tomato race 0 of *P. infestans* were chosen for evaluation of somaclonal variation. Also selfed LA 1623 were used as a control.

**Germination of seeds:** Lots of 20 seeds of each R1 somaclonal family in duplicate were space-sown in seed compost (Mon Cro-Ryt compost, Bodorgan, Angleseyl U.K.) in 8 x 8 x 7 cm pots. Seeds were covered with a thin layer of compost and were watered gently. Pots with seeds were placed in a glasshouse at a day temperature of 25 ± 2°C with natural sunlight augmented by 40 W sodium vapour lighting on a 16 h day. Subsequently, pots were watered when dry.

**Screening for late-blight sensitivity:** Isolates Ca 65 and 550 were grown on detached leaflets of potato cv Maris Bard. After seven days, sporangia were harvested by washing leaflets in water containing a 2 percent v/v potato extract. This dilute solution was used to prevent zoospores from bursting in hypotonic solution. Sporangia were incubated for half-an-hour at 4°C and two hours at room temperature for zoospore liberation. Zoospore suspensions were adjusted to $10^4$/ml for Ca 65, and 1.2 x $10^5$/ml for 550. La 1623 was resistant to 550 therefore higher concentration of this isolate was used. Seedlings (two weeks old) were sprayed lightly with distilled water then spray-inoculated with zoospore suspensions of Ca 65 or 550 until run-off. Pots of inoculated seedlings were incubated within clear plastic boxes (Propagator 52 x 35 cm Sunkey Ltd, Bulwell, Nottingham, U.K.). Each of these propagators contained 16 pots representing 16 R, families arid two pots representing two controls of LA 1623. Seedlings were inoculated with isolate Ca 65 and a similar set of material representing the same families was inoculated with isolate 550. The plastic boxes were placed in a growth room maintained at 20°C with 12 h photoperiod of fluorescent light. Seedling boxes were kept moist by spraying interior surfaces with water periodically. Seedlings were evaluated for sensitivity after seven days and again after 14 days.

## Results

**Regeneration:** Two experiments were conducted to study the effect of genotype and explant source (cotyledon or hypocotyl) on regeneration. In experiment I there were five genotypes and in the experiment 11 there were six genotypes.

**Explant survival:** The patri dishes with explants were incubated and viability of explants in experiments I and II was assessed after four weeks. In an analysis of variance, the number of surviving explants in response to various genotypes and explant sources gave highly significant interactions at the ($p < 0.01$) level in both the experiments. Significant differences in surviving explants were observed among the five and six tomato genotypes in experiments I

and II respectively (Table 1 and 2). In both the experiments a significantly higher number of explants survived when the cotyledon was used as an explant source (Table 1 and 2). With the exception of one explant of Nova in experiment II, all cotyledonary explants survived. However, survival response was variable in different genotypes when the hypocotyl was used as an explant source. In both experiments Nova, LA 1456 and LA 1623 hypocotyl survival was poor. However survival in LA 1456 was improved when younger seedlings were used in experiment II. In LA 1546 and FMX-93 most of the hypocotyl explants survived.

**Shoot Regeneration after four weeks:** Explant tissue produced little callus which developed shoots within four weeks of primary explant culture. Explants were examined at this time with the aid of a dissecting microscope and the number of shoots counted. Analysis of variances for shoot-forming capacity in the two experiments indicated that the differences in the genotypes, explant sources and genotype/explant interactions were highly significant at the ($p<0.01$) level. In both the experiments, cotyledon explants were significantly more productive than hypocotyls (Table 1 and 2). Significant quantitative differences in shoot-forming capacity were observed among the five and six tomato genotypes in experiments I and II respectively (Table 1 and 2). In both experiments, the two genotypes with the lowest shoot-forming capacity (less than one per living explant) were Nova and Rockingham and the high shoot-forming genotypes (more than one) were LA 1546, LA 1623 and LA 1456. The lower shoot regeneration of LA 1456 in experiment I was increased by using younger seedlings in experiment II. In genotype/explant interactions cotyledonary explants. produced more shoots than those from hypocotyls in all genotypes except LA 1546 in which hypocotyl explants were superior in producing shoots.

**Shoot Regeneration after eight weeks:** The number of shoots whose leaflets were visible to the naked eye were counted after eight weeks. In an analysis of variance differences due to explant source and the interaction between genotypic and explant source in shoot-forming capacity after eight weeks were highly significant ($p<0.01$) level. In both experiments cotyledons had significantly greater shoot-forming capacities than those of hypocotyls. (Table 1 and 2). Significant genotypic differences in the ability to regenerate shoots were observed among the five and six tomato accessions in experiments I and II respectively (Table 1 and 2). The tomato accessions may be classified based on the maximum average number of shoots produced per surviving explant. LA 1546, LA 1623, LA 1456 and FMX-93 were high-frequency shoot regenerating genotypes (more than two) and the remaining accessions exhibited low shoot regenerations in both the experiments. In experiment I the lower shoot regeneration of LA 1456 was improved by using younger seedlings in experiment II. Most of the genotypes in the high shoot-forming group had significant greater shoot-forming capacities than those in the shoot-forming group. Of the four high-frequency shoot regenerating genotypes, ' LA 1623' regenerated sho, most readily. Shoots were often observed after only two weeks of culture, in contrast to the three and four weeks period for other accessions. In genotypetexpli interactions cotyledon explantino produced more shoots the those of hypocotyls in all accessions except LA 1546 which hypocotyl was superior in producing shoots experiment II but not in experiment 1.

**Root formation:** Shoots produced by explants were exist after eight weeks and rooting was induced within 10 to days when the shoots were transferred to MS medium containing no growth hormones.

After four weeks on this medium the number of shoot forming roots and the number of roots per shoot was recorded. Genotypic differences in the amount of rooting were observed in both the experiments. In experiment Rockingham produced very small and slow-growing shot and these did not form any roots and hence no plants. The tomato genotypes LA 1623, LA 1546, LA 1456 and FM 93 exhibited the highest rooting. Nova had the lower rooting ratings. Variation was also shown, by individiual genotype in their rooting capacity from the cotyledon versus hypocotyl explants. In general the cotyledon explain showed a greater rooting potential in terms of percentage of shoots forming roots and number of roots per shoot Hypocotyl explants of Nova arid LA 1623 in experiments, LA 1456 in experiment I and FMX-93 experiment it produced shoots which appeared abnormal and rooting could not be induced and hence no plants was produced.

**Somaclonal variation**

**Early mutants:** These refer to phenotypes first observed the selfed progeny (R,) of regenerated plants and scored the young-seedling stage. Variant phenotypes affected early functions, such as small seedlings and seedling lenght (albino) were found in the R, seedlings two weeks after sowing. For example, all plants in somaclone No. 115 was seedling lethals (albino). Also regeneration was effectiv inducing an abnormal leaf morphology similar to "pot leaf". For example, all plants in somaclone No. 138, 1 173, 178, 113, 114, 147, 138, 92, 125, 137, 111, 1 101 and 99 had abnormal leaf morphology at an early stage. However, older leaves in all plants were wild-type. Of the 200 plants ($R_1$) raised from five genotypes only showed obvious variation, a variegated phenotype; no were produced from this plant.

**Late-blight sensitivity:** R, seedlings from selfed plant I' each of the 48 somaclonal families were tested. resistance to isolate 550 (Table 3). Seven days inoculation, seedlings of all control and R, families shop

Table 1: Effect of genotype and explant source on survival of explant and on number of shoots per explant in tomato tissue culture in experiment I

| | Number of surviving explants (mean) | | Number of shoots per surviving explant after | | | |
|---|---|---|---|---|---|---|
| | | | Four weeks (mean) | | Eight weeks (mean) | |
| | Original | Transformed | Original | Transformed | Original | Transformed |
| Genotype | | | | | | |
| FMX-93 | 4.00 | 3.99a | 1.50 | 1.46c | 2.31 | 2.22c |
| LA 1546 | 4.00 | 3.99a | 3.20 | 3.18a | 6.25 | 6.22a |
| LA 1623 | 3.13 | 3.00b | 2.53 | 2.32b | 4.78 | 4.22b |
| Nova | 3.00 | 2.85b | 0.41 | 0.36d | 0.63 | 0.53e |
| LA 1456 | 2.00 | 1.52c | 1.50 | 1.16c | 2.03 | 1.72d |
| Explant source | | | | | | |
| Hypocotyl | 2.45 | 2.16b | 1.49 | 1.16b | 2.05 | 1.72b |
| Cotyledon | 4.00 | 3.99a | 1.70 | 2.06a | 2.35 | 4.08a |

Any two means in a column not sharing a common letter different significantly at the 5 percent probability level. Figures are based on transformed data

Table 2: Effect of genotype and explant source on survival of explant and on number of shoots per explant in tomato tissue culture in experiment II

| | Number of surviving explants (mean) | | Number of shoots per surviving explant after | | | |
|---|---|---|---|---|---|---|
| | | | Four weeks (mean) | | Eight weeks (mean) | |
| | Original | Transformed | Original | Transformed | Original | Transformed |
| Genotype | | | | | | |
| FMX-93 | 3.63 | 3.58b | 1.00 | 0.94c | 1.34 | 1.30d |
| LA 1546 | 3.88 | 3.87a | 3.09 | 3.00a | 4.72 | 4.56a |
| LA 1623 | 2.38 | 2.09c | 2.09 | 1.69b | 3.25 | 2.37c |
| Nova | 2.25 | 2.00c | 0.53 | 0.43d | 0.72 | 0.63e |
| LA 1456 | 3.63 | 3.58b | 2.78 | 2.72a | 3.53 | 3.46b |
| Rockingham | 3.75 | 3.74ab | 0.82 | 0.66cd | 1.88 | 1.68d |
| Explant source | | | | | | |
| Hypocotyl | 2.58 | 2.39b | 1.36 | 0.99b | 1.84 | 1 .54b |
| Cotyledon | 3.92 | 3.91a | 2.18 | 1.98a | 3.21 | 2.90a |

Any two means in a column not sharing a common letter different significantly at the 5 per cent probability level

a hypersensitive response to 550 (necrotic flecks). After 14 days, the cotyledons and sometimes the true leaves of a small proportion of seedlings from 15 of the $R_1$ families showed non-sporulating lesions. Control seedlings (approximately 120) did not show any lesion development. A considerable range of variation was recorded for sensitivity to isolate Ca 65 (Table 4). In the control, 2 per cent plants were surviving after one week. However, all seedlings in somaclonal families No. 92, 98, 102, 113, 114, 127, 135, 137, 138, 139, 147, 148 and 178 collapsed and died by day seven. Other families appeared to have a sensitivity close to that of the control families as, like the fatter, they had a few surviving seedlings at seven days which died before 14 days. In six $R_i$ families, one or two seedlings survived at 14 days and in one family (from somaclone 123) nine of the 20 seedlings survived at 14 days.

## Discussion

**Regeneration:** Genotypes were quantitatively different in shoot and root-forming capacity from cotyledon and hypocotyol explants. Tomato genotypes LA 1623, LA 1456, LA 1546 and FMX-93 had a higher frequency of shoot regeneration and root formation than Nova and Rockingham. In previous tomato studies, a substantial influence of genotype on shoot and callus induction was reported by many authors (Padmanabhan *et al*., 1974; Kartha *et al*., 1976; Tal *et al*., 1977; Ohki *et al*., 1978; Meredith, 1979; Frankenberger *et al*., 1981; Kurtz and Lineberger, 1983; Zelcer *et al*., 1984). Genotypic differences may be related to variations in endogenous hormone levels (Norstog, 1970).

In these regeneration studies a strong interaction between the explant source and genotype was shown. Shoot

formation by cotyledon explants was found to be superior to shoot formation by hypocotyl explants in all genotypes except LA 1546 in which the hypocotyl was superior in producing shoots. This is in general agreement with previous observations in tomato (Frankenberger *et al*., 1981; Locy, 1981; Totchell and Binns, 1986). in accession LA 1456, explants derived from the younger seedlings of experiment II were shown to regenerate more shoots than those from older seedlings (Table 1 and 2). Generally, competence for organogenesis is thought to be closely related to the developmental stage of the donor tissue in many different plant species (Williams and Maheswaran, 1986). The difference in shoot-forming capacity among explants may be due to uneven distribution of endogenous growth regulators. Aung *et al*. (1975) reported an accumulation of auxin-like substance in the cotyledon and basal part of the hypocotyl of tomato. Further, it is well known that cytokinins are synthesized in the root, so, there may be an uneven distribution of these substances in the hypocotyl. Thus the genetic basis of variability in tissue culture response and morphogenesis is most likely due to differences in hormone metabolism within the explant which is established by the level of gene expression for individual hormones by genotype.

The results of these experiments confirm the importance of genotype and explant source in regeneration studies. If a genotype is to be a good candidate for somatic cell gene investigations, it is essential that we be able to regenerated whole plants from plant tissue culture. Future studies be focused on the promising genotypes recognized in the work. The identified genotypes could be especially USE when very efficient regeneration is required, which is the case when relatively rare events are selected for, such somatic hybrids, rare mutants, transformants, gene tagging Regeneration in recalcitrant genotypes like Nova Rockingham may be improved by using different concentrations or types of growth regulators or different basal media.

**Somaclonal variation:** Somaclonal variation present regenerants may reflect either pre-existing cellular gene differences in explants or physiological, epigenetic and genetic variation induced by tissue culture, as has be extensively discussed by Larkin and Scowcroft (1983). These results add further strength to the proposed use somaclonal variation to recover, at high frequency, no genetic variability from existing crop cultivars.

Regeneration from *in vitro* culture led to a higher number mutations, in the case of both early mutation and late-bling resistance. These mutations affect developmental function such as leaf shape, seedlings height and lethal seedling (albino) in this study. There is frequent recovery of mutant

Table 3: Reaction of somaclonal families and LA 1623 to zoospores of isolate 550 observed after two weeks.

| Clone No. | No of plants with lesion (%) | Clone No. | No. of plants with lesion (%) |
|---|---|---|---|
| Control* | - | 127 | - |
| 80 | - | 128 | 3(15) |
| 90 | - | 129 | - |
| 92 | - | 131 | 2(10) |
| 93 | - | 135 | - |
| 97 | 3(15) | 137 | 1(5) |
| 98 | - | 138 | - |
| 99 | - | 139 | - |
| 100 | 2(10) | 140 | - |
| 101 | 2(10) | 141 | - |
| 102 | - | 142 | - |
| 103 | - | 143 | - |
| 104 | - | 147 | - |
| 105 | - | 148 | - |
| 108 | - | 150 | - |
| 109 | - | 154 | - |
| 1 1 1 | - | 155 | 2(10) |
| 112 | 3(15) | 163 | 2(10) |
| 113 | 3(15) | 164 | 3(15) |
| 114 | 2(10) | 169 | - |
| 115 | - | 172 | 2(10) |
| 116 | - | 173 | - |
| 123 | - | 174 | - |
| 124 | - | 178 | 3(15) |
| 125 | 4(20) | | - |

*120 LA 1623 Plants; - = hypersensitive reaction; () = percent

Table 4: Reaction of somacional families and LA 1623 to zoospores of isolate Ca 65 observed after one and two weeks.

| Clone | After one week | | After two weeks | |
|---|---|---|---|---|
| | No.of plants surviving | Percentage of survival | No. of plants surviving | Percentage of survival |
| Control' | 24 | 20 | 0 | 0 |
| 80 | 4 | 20 | 0 | 0 |
| 90 | 3 | 15 | 0 | 0 |
| 92 | 0 | 0 | 0 | 0 |
| 93 | 1 | 5 | 1 | 5 |
| 97 | 1 | 5 | 1 | 5 |
| 98 | 0 | 0 | 0 | 0 |
| 99 | 1 | 5 | 0 | 0 |
| 100 | 1 | 5 | 0 | 0 |
| 101 | 2 | 10 | 0 | 0 |
| 102 | 0 | 0 | 0 | 0 |
| 103 | 1 | 5 | 0 | 0 |
| 104 | 3 | 15 | 0 | 0 |
| 105 | 5 | 25 | 0 | 0 |
| 108 | 3 | 15 | 0 | 0 |
| 109 | 4 | 20 | 0 | 0 |
| 111 | 1 | 5 | 0 | 0 |
| 112 | 8 | 40 | 0 | 0 |
| 113 | 0 | 0 | 0 | 0 |
| 114 | 0 | 0 | 0 | 0 |
| 115 | 1 | 5 | 0 | 0 |
| 116 | 3 | 15 | 0 | 0 |
| 123 | 12 | 60 | 9 | 45 |
| 124 | 5 | 25 | 0 | 0 |
| 125 | 2 | 10 | 0 | 0 |
| 127 | 0 | 0 | 0 | 0 |
| 128 | 7 | 35 | 2 | 10 |
| 129 | 6 | 30 | 0 | 0 |
| 131 | 3 | 1 5 | 0 | 0 |
| 135 | 0 | 0 | 0 | 0 |
| 137 | 0 | 0 | 0 | 0 |
| 138 | ❑ | 0 | 0 | 0 |
| 139 | 0 | 0 | 0 | 0 |
| 140 | 4 | 20 | 0 | 0 |
| 141 | 3 | 1 5 | 0 | 0 |
| 142 | 3 | 15 | 0 | 0 |
| 143 | 2 | 10 | 0 | 0 |
| 147 | 0 | 0 | 0 | 0 |
| 148 | 0 | 0 | 0 | 0 |
| 150 | 4 | 20 | 0 | 0 |
| 154 | 2 | 10 | 0 | 0 |
| 155 | 5 | 25 | 1 | 5 |
| 163 | 6 | 30 | 0 | 0 |
| 164 | 8 | 40 | 2 | 10 |
| 169 | 4 | 20 | 1 | 5 |
| 172 | 1 | 5 | 0 | 0 |
| 173 | 2 | 10 | 0 | 0 |
| 174 | 4 | 20 | 0 | 0 |
| 178 | 0 | 0 | 0 | 0 |

*120 LA 1623 plants.

in the $R_1$ generation (Shepard *et al*., 1980; Gavazzi *et al*., 1987). Evidence is lacking on their origin, however whatever the basis of this phenomenon, this is not new in the literature (Evans and Sharp, 1983; Larkin and Scowcroft, 1983).

One of the major limitations to utilizing somaclonal variation in a breeding programme is the identification of desired mutant phenotypes. In the work described herein, a rapid and efficient protocol has been used for the recovery of somaclones from a selected donor genotype. The criterion of resistance used in this experiment for selection of a total of 17 seedlings was that most survived zoospore ($10^4$/ml) inoculation of Ca 65 under environmental conditions which led to the death of the seedlings of LA 1623 control. The differences in disease response between control and resistant somaclones may be attributable primarily to mutations as they occur spontaneously and apparently at very high rates in cell cultures (Evans and Sharp, 1983). Hoswever, seedling survival may have been due to some seedlings accidentally receiving a smaller zoospore inoculum. Repetition of the experiment might show that the same families have increased resistance or that other families showed resistance. if the former results were found, this would provide evidence of a genetic basis for increased seedling survival. Testing detached leaflets of surviving plants could provide more evidence for or against genetic resistance. There was little variation for sensitivity to isolate 550. Resistance is specific or non-specific or these may be crossed with susceptible lines to determine the inheritance pattern.

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