Theor Appl Genet (1999) 98: 299–304 © Springer-Verlag 1999

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In vitro versus in vivo genetic divergence in potato

Received: 15 September 1997 / Accepted: 15 July 1998

Abstract The objective of this study was to compare the genetic divergence pattern in potato (*Solanum tuberosum* L.) under in vitro and in vivo conditions. Twenty two potato genotypes were evaluated for ten morphological characters under four in vitro conditions, and for 20 characters under four in vivo seasons. Mahalanobis' generalized intra- and inter-group genetic distances, and the distribution of genotypes into different clusters, led to the same conclusions under both in vitro and in vivo conditions: (1) genetic diversity was not related to geographic diversity, (2) genetic distances were higher between Tuberosum and Andigena than within Tuberosum and Andigena, and (3) present-day Indian varieties have more resemblance to Tuberosum than to the Andigena group. The in vitro approach was more effective than the in vivo approach for differentiating the genotypes *per se*, although its effectiveness for cross prediction is known to be low.

Key words Genetic divergence · Mahalanobis D^2 statistic · In vitro · In vivo · *Solanum tuberosum* · Potato

Introduction

The concept of "genetic distance" has been of considerable utility in many contexts and especially in differentiating genotypes at the genome level. Principal component analysis, cluster analysis, or Mehalanobis' D^2 statistics are the commonly used quantitative measures for this purpose (Mahalanobis 1936; Spark 1973; Singh and Chaudhary 1977; Dillon and Goldstein 1984). Of these approaches Mahalanobis' generalized distance (D^2 statistic) has been extensively used in several crops. Genetic divergence can be estimated from geographical or pedigree information, which does not require observations to be made on the plant material, or from phenotypic characters, biochemical or molecular markers, which require that the plant material is evaluated. The best source of information for measuring genetic divergence is uncertain; usually different estimates are not related (Lefort-Bunson et al. 1986; Damerval et al. 1987; Loiselle et al. 1991).

Responses of potatoes to tissue culture are genetically controlled (Henry et al. 1994; Gopal 1996). Genotypic variability is present for various in vitro quantitative characters in potato (Maroti et al. 1980; Amirouche et al. 1985; Miller et al. 1985; Gopal 1996). But this variability has rarely been used in genetic divergence studies. In potato, the exchange of germplasm as in vitro plantlets has become a routine procedure. Evaluation of germplasm in vitro, before it is ready to be transferred to the fields, will help in an early evaluation of the material received. The study presented here reports the genetic divergence in potato based on in vitro and in vivo morphological characters, and their comparison.

Materials and methods

This study was conducted at the Biotechnology Centre, Punjab Agricultural University, Ludhiana (31°N, 75°E, 230 m above sea level), during 1993–96. A random sample of 22 potato genotypes (see Table 3) was drawn from the Indian Potato Breeding Programme. Two of these, EX/A680-16 and EX/A723, belong to *Solanum tuberosum* ssp. *andigena* and the rest to ssp. *tuberosum*. Accessions CP1710 and CP2132 are varieties "Kerr Pondy" from France, and "Tollocan" from Peru, respectively; whereas JH222 ('Kufri Jawahar') and PJ376 ('Kufri Ashoka') are recently released Indian varieties. The remaining genotypes are advance generation clones of Indian origin.

Communicated by H. C. Becker

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Table 1 In vitro treatments used for the evaluation of genotypes

Treatment	Media	Incubation conditions
MT1A	40 ml of solid MS media with 8% sucrose, no hormones	16-h day (3000–4000 lux; 28 ± 2°C); 8-h night (25 ± 2°C)
MT1B	40 ml of solid MS media with 8% sucrose, no hormones and, after 60 days of culture, addition of 20 ml of liquid MS media with 8% sucrose and 10 mg/l BAP	16-h day (3000–4000 lux; 28 ± 2°C); 8-h night (25 ± 2°C)
MT2A	40 ml of solid MS media with 8% sucrose, no hormones	10-h day (500–1000 lux; 20 ± 2°C); 14-h night (18 ± 2°C)
MT2B	40 ml of solid MS media with 8% sucrose, no hormones and, after 60 days of culture, addition of 20 ml of liquid MS media with 8% sucrose and 10 mg/l BAP	For initial 60 days: 16-h day (3000–4000 lux; 28 ± 2°C) and 8-h night (25 ± 2°C); For remaining period: 10-h day (500–1000 lux; 20 ± 2°C) and 14-h night (18 ± 2°C)

In vitro experiments

The genotypes were evaluated by establishing plantlets from single nodal sections (0.5–1.0-cm length) dissected from etiolated sprouts, and aseptically cultured in 150 × 25 mm test tubes, one segment per test tube. Each test tube contained 12–15 ml of semi-solid (7 $g l^{-1}$ agar) MS basal medium (Murashige and Skoog 1962) with 3% sucrose. The cultures were incubated for a 16-h photoperiod of 3000 lux at 28 ± 2°C day and 25 ± 2°C night temperatures. Six-to-eight week-old plantlets, thus established, were subcultured under four conditions (Table 1), bearing in mind the objectives of another study (Gopal 1996). All the experiments were conducted in a randomised complete block design with four replications, each comprising of four culture vessels (300-ml jam jars) and four nodal sections/jam jar.

Data were recorded for ten characters on all plantlets in each treatment. Foliage characters were recorded at the onset of senescence, and microtuber characters at maturity. The characters were: plantlet vigour (score 1–3: 1, good; 3, poor), plantlet height (cm), number of nodes per plantlet (on the longest stem only), average internode length (cm), extent of branching (score 1–3: 1, profuse; 3, scanty), extent of rooting (score 1–3: 1, good; 3, poor), leaf size (score 1–3: 1, big; 3, small), microtuber yield per plantlet (mg), microtuber number per plantlet, and average microtuber weight (mg).

In vivo experiments

The above genotypes were also evaluated in two spring (January to May) and two autumn (October to January) seasons in the field. All trials were laid out in a completely randomised block design in short rows of five tubers each at recommended intra- and inter-row distances of 20 cm and 60 cm, respectively. Normal manure and cultural schedules were followed.

Data were recorded for 20 characters. Foliage characters were recorded at full growth (80 days after sowing) on five competitive plants per plot, and tuber characters on a plot basis at maturity (120 days after planting). Leaf characters were recorded on the fourth leaf from the top. The characters were: plant vigour (score 1–5: 1, very high; 5, very poor), stem habit (score 1–3; 1, erect; 2, intermediate; 3, prostrate), leaf colour (score 1–3: 1, pale green; 2, green; 3, dark green), stem pigment (score 1–3: 1, absent; 2, present at stem base only; 3, present at base as well as in upper parts), number of main stems per plant, plant height (cm), number of nodes per plant (on the longest stem only), internode length (cm), leaf length (cm), leaf width (cm), leaf index (ratio: leaf length/leaf width), leaflet length (cm), leaflet width (cm), leaflet index (ratio: leaflet length/leaflet width), days to foliage senescence, tuber yield per plant (g), tuber number per plant, average tuber weight (g), number of eyes per tuber and general impression based on overall tuber characters (score: 1–5: 1, very high; 5, very low).

Statistical methods

Before conducting analysis, the normality of data, particularly for variables scored on a scale, was tested by determining the relationship between the mean and the variance/standard deviation. The absence of any such relationship indicated that the data were normally distributed. Two sets of multivariate analysis (Morrison 1976), one pooled over four in vitro conditions and the other pooled over four in vivo conditions, were conducted in a randomised complete block design to calculate variances and co-variances. From these estimates, a dispersion matrix was prepared. Using the 'V' statistic, which in turn utilizes Wilk's λ criterion (Rao 1952), a simultaneous test of the significance of the difference between the mean values of the correlated variables of characters was carried out. The correlated variables were transformed to uncorrelated ones using the pivotal condensation method (Singh and Chaudhary 1977) for simplifying the computation of Mahalanobis (1936) distances (D^2), which were computed as the sum of squares of the differences between pairs of means of genotypes. Computer software SPAR-1 (IASRI, New Delhi) was used for the above steps.

For all the combinations [i.e. $n(n-1)/2 = 231$, where n is the number of genotypes, 22 in the present case] each character was ranked on the basis of the difference in the means of a pair of genotypes (i.e. the di value). Rank 1 was given to the highest mean difference and rank p (p = number of characters) to the lowest mean difference. The per-cent contribution based on the number of times a character appeared first in ranking was calculated. Rank totals, obtained by a character in different parental combinations, were also computed.

The grouping of genotypes based on D^2 values was done by Toucher's method (Singh and Chaudhary 1977). For this purpose genotypes were arranged in order of their relative distances (D^2 values) from each other. Two genotypes having the smallest distance from each other were considered first to form cluster I, to which a third genotype having smallest average D^2 value from the first two genotypes was added. This procedure of adding another genotype to a cluster was continued until the increase in the average D^2 value by adding a genotype was equal to, or higher than, the maximum D^2 value shown by a genotype to the nearest genotype. Similarly, subsequent clusters were formed and the process was continued untill all the genotypes were included in one or another cluster.

Results

In vitro experiments

Pooled analysis over four treatments indicated highly significant differences among genotypes for all

Table 2 Contribution by different characters to total genetic divergence

Based on in vitro study

Characters	Appeared first in ranking		Rank total
	Number of times	Per-cent times	
Plantlet vigour	3	1.30	1382
Plantlet height	72	31.17	823
Number of nodes	6	2.59	1467
Internode length	44	19.05	1026
Leaf size	7	3.03	1291
Extent of branching	1	0.43	1707
Extent of rooting	11	4.76	1476
Microtuber yield	53	22.94	955
Microtuber number	28	12.12	1156
Average microtuber wt.	6	2.60	1422

Based on in vivo study

Characters	Appearig first in ranking		Rank total
	Number of times	Per-cent times	
Plant vigour	17	7.35	2090
Plant height	22	9.52	1738
Number of nodes	1	0.43	2531
Internode length	0	0.00	3238
Number of stems	0	0.00	3073
Leaf length	2	0.87	2409
Leaf width	9	3.89	2088
Leaf index	1	0.43	3490
Leaflet length	0	0.00	2898
Leaflet width	1	0.43	2669
Leaflet index	0	0.00	3204
Leaf colour	9	3.89	2234
Stem habit	0	0.00	2354
Stem pigment	40	17.3	1664
Days to maturity	25	10.82	1649
Tuber yield	1	0.43	2514
Tuber number	17	7.35	2983
Average tuber wt.	4	1.73	2633
Number of eyes	43	18.61	1715
General impression	39	16.89	1336

the characters studied. The calculated 'V' statistic (3643.21) was highly significant (chi-square for $P<0.001 = 277.52$, for $210df$). The maximum contribution towards total genetic divergence was by plantlet height, followed by microtuber yield per plantlet (Table 2). Internode length and the number of microtubers per plantlet were also of considerable importance in estimating the divergence values. Other characters were of little importance, contributing only 0.4–3.0% of the total divergence, individually. However, cumulatively they accounted for 14.7% of the total contribution. The rank total (Table 2) also reflected the same trend. In general, the lower rank total, the higher was the contribution to divergence.

The 22 genotypes were grouped into seven clusters on the basis of D^2 values (Table 3). Cluster I was the largest. It included six genotypes, and was followed by cluster III with five genotypes and cluster II with four genotypes. Clusters IV, V and VI each had two genotypes. Cluster VII was the smallest with only one genotype. The two Andigena accessions, i.e. EX/A680-16 and EX/A723, fell in clusters VI and VII, respectively. These two clusters were more closely related to each other than with any of the remaining clusters (Table 4), which included the rest of the accessions which belonged to the group Tuberosum.

In vivo experiments

The analysis of variance indicated highly significant differences among genotypes for all 20 characters used for grouping. The calculated 'V' statistic (2890.97) was highly significant (chi-square for $P<0.001$ is 513.78, for $420df$). The maximum contribution to total genetic divergence (Table 2) was by the number of eyes (18.61%), followed by stem pigment (17.3%), general impression (16.89%), days to maturity (10.82%), plant height (9.52%), tuber number (7.35%), plant vigour (7.35%), leaf colour (3.89%), leaf width (3.89%) and average tuber weight (1.73%). The individual contributions of the remaining characters were negligible, some of them having a nil contribution. If only rank totals are considered, the general impression appeared to be the largest contributor followed by days to maturity, stem pigment, number of eyes and plant height, respectively.

All the genotypes were grouped into five clusters (Table 3). Cluster I was the largest having 15 (68.18%) genotypes. Cluster II had three genotypes and cluster III had two. Clusters IV and V each consisted of individual genotypes. The two Andigena accessions EX/680-16 and EX/A723 were grouped in cluster II. The only other genotype in this cluster was JR465. All other clusters had Tuberosum accessions only. In general, clusters with Tuberosum accessions were not as divergent from each other as they were from cluster II having the Andigena accessions (Table 4).

In vitro versus in vivo experiments

If the characters which were used for both the in vitro and in vivo studies are considered, plantlet height and the number of tubers appeared to be the major contributors to genetic divergence in both the cases. Tuber yield and internode length which were important in the in vitro studies, were however, of little importance in studying genetic divergence in vivo. The clustering pattern (Table 3) showed that both the in vitro and the in vivo behaviour of various characters could distinguish the pure Andigena types (EX/A680-16 and EX/A723) from the other genotypes, because Andigena accessions were grouped in clusters separate

Table 3 Distribution of 22 genotypes in different clusters

Based on in vitro study

Cluster	Number of geonotypes	Genotype(s)
I	6	E4451, JH222, JR465, MS81-152, MS82-638, SLB/K23
II	4	JN1501, MS78-56, MS79-34, RG1197
III	5	F1277, JTH/C107, MS78-46, MS80-758, MS84-1169
IV	2	CP2132, JE812
V	2	AB455, CP1710
VI	2	EX/A680-16, PJ376
VII	1	EX/A723

Based on in vivo study

Cluster	Number of genotypes	Genotype(s)
I	15	CP1710, CP2132, E4451, F1277, JE812, JH222, JTH/C107, MS78-46, MS78-56, MS79-34, MS80-758, MS81-152, PJ376, RG1197, SLB/K23
II	3	JR465, EX/A680-16, EX/A723
III	2	MS82-638, MS84-1169
IV	1	JN1501
V	1	AB455

Table 4 Average intra- and inter-cluster D^2 values

Based on in vitro study

Clusters	I	II	III	IV	V	VI	VII
I	22.15	70.42	49.10	138.44	65.81	135.22	155.68
II		37.24	145.89	202.66	78.36	280.43	279.83
III			34.18	84.53	78.66	108.58	189.31
IV				17.63	187.65	116.92	155.53
V					30.08	232.68	288.58
VI						54.89	106.46
VII							0.00

Based on in vivo study

Cluster	I	II	III	IV	V
I	40.74	158.59	49.55	98.87	123.40
II		49.54	154.82	181.96	98.84
III			47.70	153.78	109.27
IV				0.00	174.88
V					0.00

from those in which most of the Tuberosum accessions were classified. There was, however, low commonality with regard to the grouping of Tuberosum genotypes in the in vitro and in vivo studies. Thirteen out of fifteen genotypes of cluster I of the in vivo study were grouped into the first four clusters of the in vitro study. The remaining two genotypes, viz. CP1710 and PJ376, of cluster I of the in vivo study were in clusters V and VI, respectively, of the in vitro study. MS82-638 and MS84-1169 which were together in cluster III of the in vivo study were in two different clusters, i.e. I and III, respectively, in the in vitro study. Such variation in grouping was also observed for some other genotypes. Genotypes from different countries and from different regions of India were grouped together in the same cluster, both in the in vitro and in vivo experiments.

Discussion

The objective of this study was to compare the genetic divergence pattern under in vitro and in vivo conditions. Since the grouping was done on the basis of morphological characters, whose performance is influenced by environment, data recorded over four

varying environments both for the in vitro and the in vivo conditions, were used. The number and type of characters employed for grouping were different in in vitro and in vivo studies (Table 2). However, some characters were common to both.

Gaur et al. (1978), who studied the genetic divergence in potato under in vivo conditions, reported that characters for which no selection pressure was applied during the evolution of the present-day varieties were more important to genetic divergence. The non-importance of tuber yield, and importance of the number of eyes and stem pigment to genetic divergence, as observed in the present study, support their conclusion.

In South America, the site of origin of potatoes, the cultivated tetraploid potato is represented by *S. tuberosum* groups Tuberosum and Andigena. Of these Andigena is found at high altitudes in the Andes, extending from Colombia to North Argentina, whereas Tuberosum occurs at low altitudes, in temperate Chile. The earliest potato introductions to Europe were of the Andigena type, since the relics of old European potato varieties found in Lesotho (Van Der Plank 1946) and in India (Swaminathan 1958) resemble the Andigena group in morphological characters and photoperiodic response. From this basic material, the European and American commercial varieties were evolved by selection for tuber yield under long photoperiodic conditions (Glendinning 1975). In contrast, the Indian varieties were bred either for their yielding ability under long, as well as short, photoperiodic conditions or for their response under short photoperiods alone. The material in the present study, represent the advance breeding lines, developed by crossing Tuberosum to Tuberosum, Andigena, or wild species at some stage of the potato-breeding programmes. However, after several cycles of crossing and selection, most of them have the features of the Tuberosum group. Only two accessions (EX/A680-16 and EX/A723) in the present study were of pure Andigena. The results showed that Tuberosum and Andigena germplasm can be differentiated by D^2 statistic both under in vitro and in vivo conditions. Gaur et al. (1978) arrived at a similar conclusion in a study based on in vivo evaluations. Two exotic accessions, CP1710 and CP2132, which belong to the Tuberosum group were grouped along with the Indian lines. This shows that present-day advanced Indian breeding lines have more resemblance to the Tuberosum group rather than to the Andigena group. The clustering pattern of both the in vitro and in vivo experiments did not follow the geographic distribution of the genotypes. This confirms the report of Gaur et al. (1978) that genetic divergence is not related to geographic diversity.

Within Tuberosum the divergence was poor, as 15 out of 20 Tuberosum accessions were grouped in cluster I, in the in vivo study. The low divergence in Tuberosum may be due to their narrow genetic base. This is expected because, generally, a very limited number of parental lines are used in Indian potato breeding programmes. The grouping of the exotic accessions CP1710 and CP2132 in this same cluster (i.e. cluster I) indicates that, in general, Tuberosum has a very narrow genetic base. Simmonds (1962), Gaur et al. (1978) and Sidhu et al. (1981) also pointed to the narrow genetic base of most of the cultivated varieties, resulting from a very limited number of breeding clones which may be inter-related through common ancestors (Hougas and Ross 1956; Ross 1958). The poor progress made in the improvement of Tuberosum can, therefore, be largely attributed to a low level of diversity.

Intra-cluster distances both in the in vivo and in vitro studies (Table 4) were much lower than the inter-cluster distances. This suggests the heterogenous and homogenous nature between and within groups, respectively. Gopal (1996) reported that phenotypic differences are enlarged under in vitro as compared to in vivo conditions. In general the higher average intra- and inter-cluster D^2 values for the in vitro clusters compared with those of the in vivo clusters, as observed in the present study (Table 4), supports this observation. Due to this the 22 genotypes were grouped in seven clusters under the in vitro conditions and in five clusters in the in vivo conditions. This shows that the in vitro approach is more powerful than the in vivo in distinguishing the genomes of various genotypes. This is more so, because the in vitro study was based on only ten characters as compared to 20 in the in vivo study. The in vitro method also offers some additional advantages, such as the greater number of genotypes that can be handled in a small space and on a year-round basis, irrespective of the crop season. Further, by varying the cultural conditions and media, different environments can be created more easily under in vitro conditions than in vivo. Though the in vitro approach is desirable for differentiating the genotypes *per se*, genetic divergence under in vitro conditions was found to be less effective in predicting the in vivo cross performance (Gopal and Minocha 1997). Where the objective is to use genetic divergence for the selection of parents in breeding programmes, parents should be evaluated under the conditions in which progenies are likely to be evaluated (Gopal and Minocha 1997).

This is the first report in which in vitro characters have been used for genetic divergence studies. The results clearly show that in vitro and in vivo genetic divergence studies lead to the same conclusions, namely that:

(1) genetic diversity is not related to geographic diversity;
(2) genetic distance is higher between Tuberosum and Andigena than within Tuberosum and Andigena; and
(3) present day Indian varieties have more resemblance to Tuberosum than to Andigena.

Acknowledgments I thank Dr. J. L. Minocha for his supervision and kind guidance during the course of this study.

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